SCREENING FOR LUPUS ANTICOAGULANT ANTIBODY AND ANTICARDIOLIPIN ANTIBODY IN RECURRENT PREGNANCY LOSS

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CERTIFICATE

This is to certify that the dissertation titled "**SCREENING FOR LUPUS ANTICOAGULANT ANTIBODY AND ANTICARDIOLIPIN ANTIBODY IN RECURRENT PREGNANCY LOSS"** is the bonafide work done by **Dr.S.MAHESWARI** between December 2009 to November 2010 during her M.D, O.G., Course at Institute of Social Obstetrics and GOVT. Kasturba Gandhi Hospital, Madras Medical College, Chennai.

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DECLARATION

I **Dr.S.Maheswari** solemnly declare that the dissertation titled '**Screening for lupus anticoagulant antibody and anticardiolipin antibody in recurrent pregnancy loss'** has been prepared by me.

This is submitted to the Tamil Nadu Dr. MGR Medical University, Chennai in partial fulfillment of the rules and regulations for MD Examination in Obstetrics and Gynaecology. This has not been previously submitted by me for the award of any degree or diploma from any university.

Place : Chennai

Date : 15.12.2010

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CONTENTS

INTRODUCTION

INTRODUCTION

In this era of in vitro fertilization and genetically engineered babies, there still are certain conceptions which end before they reach the maturity causing great physical and mental agony to the parents. Though there are innumerable causes to this, immunological causes are important which account for 20-50%. (1)

All of us are products of successful conception and gestation across immunological barriers, barriers that should result in the rapid rejection of fetus. Yet the fetus is tolerated and successful enough to keep the population ever increasing. The maternal immune system is able to coordinate the dichotomous responsibilities to protect the host from pathogenic microorganisms without rejecting the antigenically foreign fetus.

Pregnancy is a unique immunological state where a natural homoeostasis exists between antigenically different tissues. One of the many initial hypotheses on the maintenance of fetal allograft was that pregnancy was associated with suppression of maternal immune response thereby allowing for fetal survival, however maintaining the immunocompetence against pathogenic microorganisms and neoplasia.

The process of implantation is quite complex and in 90% of pregnancies it achieves the right balance of hormones, cytokines and growth factors,

causing degradation of the endometrium and proliferation of the trophoblast, protecting it at the same time from maternal cell mediated immunity. Nature orchestrates a host of substances in a masterly manner to achieve this goal.

Autoimmunity is the process by which humoral or cellular response is directed against specific components of host and is the result of breakdown of host immunoregulatory mechanism to discriminate self and nonself antigens.

Lupus anticoagulant antibody(LAC) and anticardiolipin antibody (ACL)are acquired antibodies directed against phospholipids or their binding proteins, characteristically found in patients with autoimmune disorders, and considered to be an important marker for pregnancy losses and intrauterine fetal demise(2).

Recently lupus and anticardiolipin antibodies have been recognized as having a role in recurrent pregnancy loss, even in women with no clinically diagnosed autoimmune disease(3).

The association between anti phospholipid antibodies and recurrent pregnancy loss is well established (4). What is still unclear is the actual prevalence of anti phospholipid antibodies in women with unexplained recurrent pregnancy loss.

Evaluation for the prevalence of these anti bodies in patients with recurrent pregnancy loss can improve the out come in future pregnancy (5).

HISTORY

Antiphospholipid antibodies were first detected during 1906 in patients with false positive serological test for syphilis. In 1952, **Conley and Hartman** were the first to describe an in vitro circulating anticoagulant which occurred predominantly in patients with Systemic Lupus Erythematosus. In 1972, **Feinstein and Rappaport** termed the anticoagulant 'lupus anticoagulant'.

A possible association between antiphospholipid antibody and pregnancy loss was first suggested in case reports by **Nilsson et al** in 1975 and by **Soulier and Boffa** in 1980. Subsequent case series by **Lubbe et al, Lockshin et al,** and **Branch et al** confirmed this association. By the mid – 1980s, the clinical criteria for the newly named antiphospholipid syndrome (APS) were established, and pregnancy loss was included as one of the clinical features of the disorder. **Harris** in 1987 identified the Antiphospholipid Syndrome pregnancy loss criterion as fetal loss. Since that time, definitions and criteria have expanded, with the 1999 clinical and laboratory criteria proposed at the international Antiphospholipid Symposium in Sapporo, Japan identifying current consensus.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Recurrent pregnancy losses may be caused by a number of mechanisms. They can be broadly divided into those caused due to abnormalities in either the pregnancy itself or in the environment. Abnormalities in the pregnancy are mainly chromosomal, while abnormalities in the environment are grouped as anatomic, immunologic and hormonal (6).

Chromosomal abnormalities occur in about 50% of all products of conception from first trimester miscarriages, 5% of late pregnancy losses and 0.5% of live births (**Boue et al**). When products of conception from over 200 miscarriages of women with recurrent pregnancy loss were tested with chromosomal analysis, 55% were abnormal. Of interest, only 35% of women experiencing recurrent pregnancy loss after a live birth were chromosomally abnormal (7). Some pregnancy losses associated with abnormal chromosomes such as an extra chromosome (trisomy) have been reported to have a high risk of a repeating. However, if such "accidents" explained all of recurrent miscarriage, the probability of three or more miscarriages in a row resulting from "accidents" would account for 5% or less of the observed incidence of losses (8).

Anatomical lesions are lesions of uterus which if present would mechanically inhibit normal implantation and hence normal embryo and/or fetal growth. These include congenital factors (Mullerian duct anomalies) and acquired factors (Cervical incompetence, uterine synechiae, sub mucous fibroid and polyps) (9).

Hormonal (Luteal phase) defect was diagnosed by endometrial dating in the past. However the results of endometrial biopsy have failed to correlate with pregnancy outcome (10). Poly cystic ovary disease, uncontrolled Diabetes mellitus, Thyroid disorders, and hyperprolactinemia may lead to pregnancy wastage.

Immunologic mechanisms have recently been recognized as a major cause of recurrent pregnancy loss associated with the loss of chromosomally normal pregnancies. Forty – five percent of miscarriages and 95% of late pregnancy losses from women experiencing recurrent pregnancy loss are chromosomally normal. Approximately 75% of chromosomally normal preimplantation embryos fail to implant. A literature is developing which suggests a role of the immune system in the majority of these losses (11).

The end result of the immunologic processes that leads to loss of the pregnancy involves interference of the blood supply to the pregnancy.

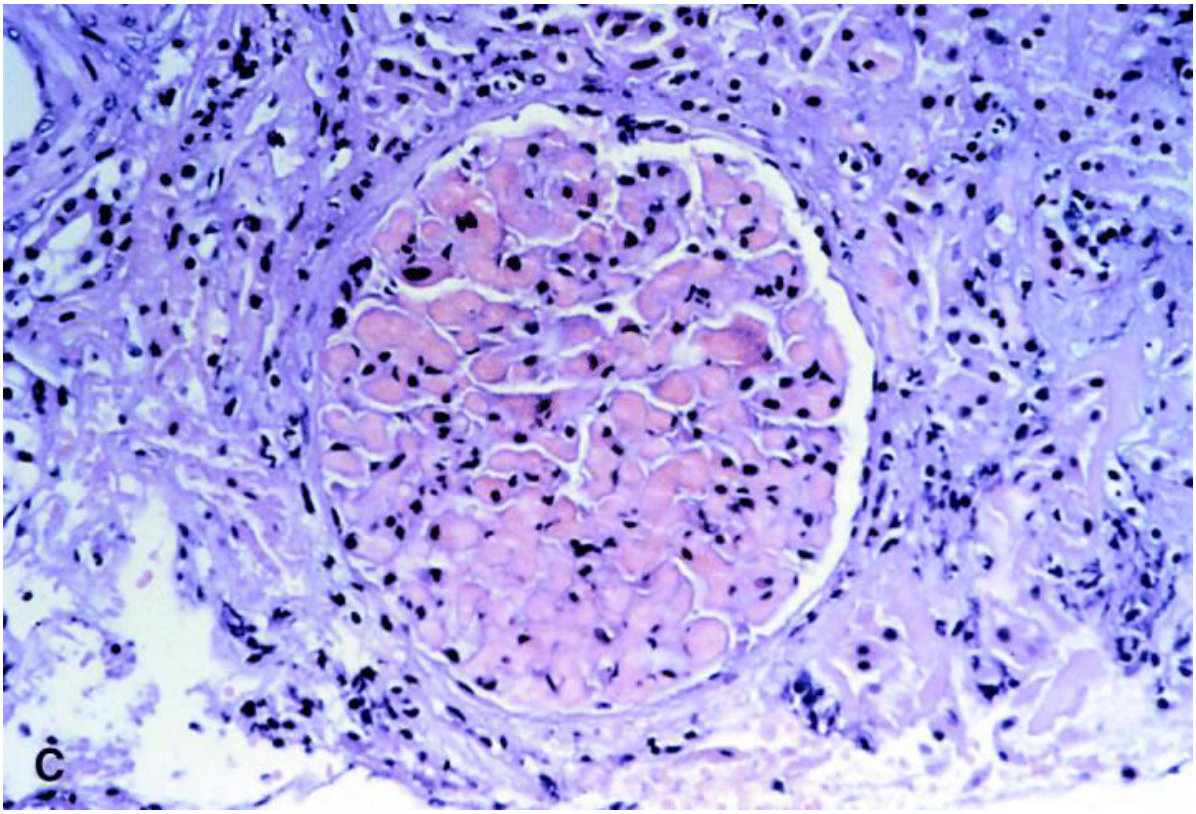
Interference of blood supply among early and late pregnancy losses is manifested by clotting of the placental/fetal vessels (12). Clotting may be caused by

- cytokines that are produced by immunological cells or
- by antiphospholipid antibodies produced by B cells or
- by a genetic predisposition contributed by thrombophilia gene.

ANTIPHOSPHOLIPID ANTIBODIES

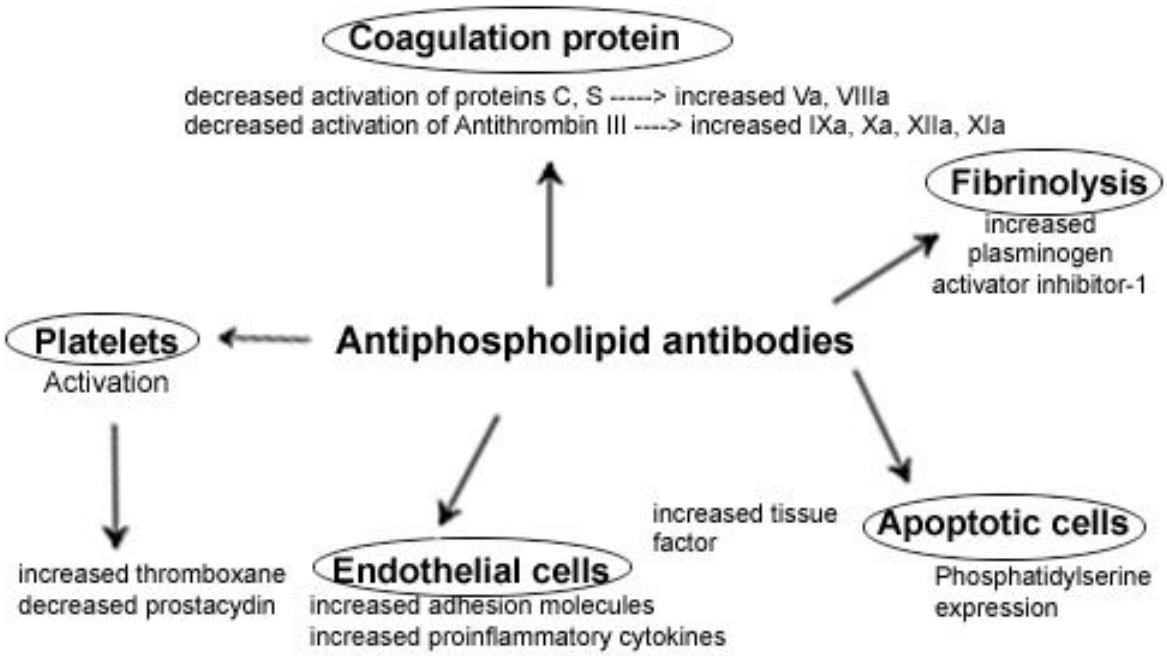
Antiphospholipid antibodies are a class of antibodies directed against phospholipids and include anticardiolipin antibody and lupus anticoagulant. Their prevalence in a general obstetric population is around 2-7%. In low-risk pregnancies, this carries a 3-9 fold risk of fetal loss, but in high – risk pregnancies they are associated with a 90% risk of further pregnancy loss (13).

Lupus anticoagulant antibody is a monoclonal antibody that reacts to the phospholipid from blood platelet membrane factor3. **Feinstein and Rappaport** (1972) introduced the term lupus anticoagulant based on the recognition that certain patients with lupus had prolonged coagulation tests and suggested some anticoagulant activity. LAC is associated with a fetal mortality



Placental Thrombosis

Pathogenetic mechanism of antiphospholipid antibodies



of 85-92%. It is present in 2-5% of normal obstetric population, 30% of patients with severe preeclampsia and 10% of non – pregnant patients with endometriosis (14).

In patients with recurrent miscarriage, lupus anticoagulant antibody is the most common, followed by IgG and IgM anticardiolipin antibodies. The lupus anticoagulant is an inhibitor of the coagulation pathway, and causes prolongation of the kaolin clotting time, which is not corrected by mixing the patient's plasma with control plasma. The association between antiphospholipid antibodies and thrombosis is stronger for lupus anticoagulant and anti beta 2 glycoprotein1 antibodies; the risk of pregnancy loss is related to anticardiolipin IgG titers (15).

PATHOPHYSIOLOGY

The antiphospholipid antibodies exert their action by binding directly to β2glycoprotein1 antibodies as in the case of anticardiolipin antibodies or to phospholipid bound prothrombin as in the case of lupus anticoagulant antibodies (16).

β2glycoprotein1 acts by phospholipid dependant anticoagulant inhibition of prothrombinase activity and adenosine di phosphate(ADP) induced platelet aggregation. Thus, it specifically inhibits the binding of coagulation factors

especially factor XII and prothrombinase to negatively charged phospholipid surfaces and prevents activation of coagulation cascade.

β2glycoprotein 1 is found in high concentration in syncytiotrophoblast and is also involved in implantation; thereby its loss results in either intervillous space thrombosis or prevents implantation.

The proposed mechanisms of pregnancy loss in Antiphospholipid antibody syndrome are,

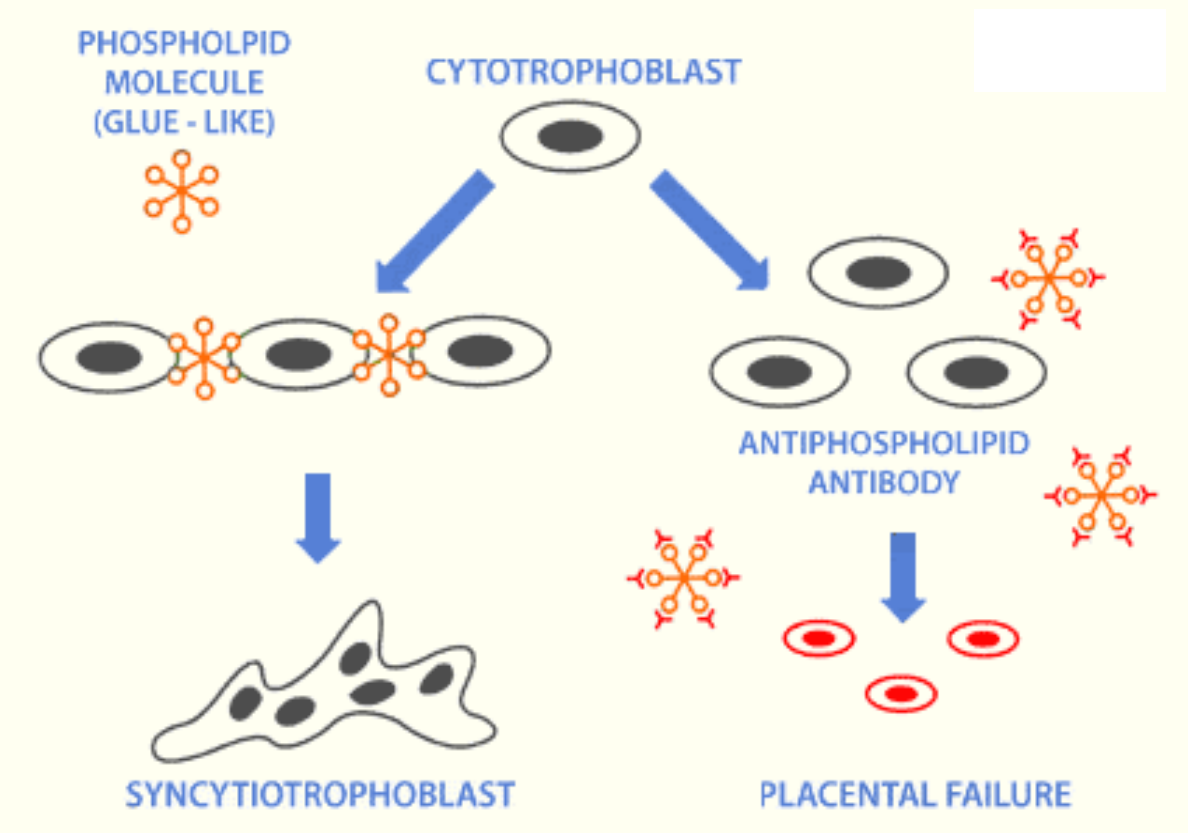
I. decreased ProstaglandinI2 and increased ThromboxaneA2 production by endothelial cells.

II. inhibition of activation of protein C and protein S pathway.

III. decreased annexin V production, inhibition of its function in placenta by antiphospholipid antibody.

IV. inhibition of heparin dependant activation of antithrombin III.

V. activation of endothelial cells and platelets, increased expression of adhesion molecules.



Mechanism of Placental Failure

PATHOLOGY

Placenta is one of the major targets of antiphospholipid antibodies. Second or third trimester fetal death is widely considered as most specific for antiphospholipid syndrome. First trimester spontaneous abortion is not uncommon provided anatomic and chromosomal causes are excluded.

These are caused by uteroplacental insufficiency, which is attributed to vasculopathy involving the terminal branches of the uterine arteries (spiral arteries). This vasculopathy is characterized by absence of normal physiologic changes in the myometrial segments of the spiral arteries underlying the placenta and accumulation of lipid laden macrophages in the intima, fibrinoid necrosis of the media and intimal fibroblastic proliferation. The worst pregnancy outcomes are associated with multifocal uteroplacental thrombosis and placental infarction, both extreme expressions of the vasculopathy (5).

THE CRITERIA FOR THE CLASSIFICATION OF ANTIPHOSPHOLIPID SYNDROME (SAPPORO, 1998) was revised in 2006.

CLINICAL CRITERIA

Vascular thrombosis:

One or more episodes of arterial, venous or small vessel thrombosis in any organ or tissue, diagnosed objectively.

Pregnancy morbidity

- One or more unexplained death of a morphologically normal fetus at or beyond 10^{th} week of gestation, normality documented by direct examination or Ultrasound or

- One or more premature births of a fetus before 34 weeks gestation because of severe preeclampsia or severe placental insufficiency or

- Three or more unexplained consecutive spontaneous abortions before the 10^{th} week with maternal anatomic, hormonal and paternal karyotype abnormalities excluded.

LABORATORY CRITERIA

I. Anticardiolipin antibody of IgG and / or IgM isotype in serum or plasma present in medium or high titer on 2 or more occasions at least 12 weeks apart by standardized ELISA for β2glycoprotein1 inhibitor dependant ACL

II. Lupus anticoagulant present in serum or plasma on 2 or more occasions at least 12 weeks apart detected according to the gudelines of the International society on Thrombosis and Haemostasis.

III. Anti beta 2 glycoprotein 1 antibody of IgG and / or IgM isotype in serum or plasma > 99 percentile present on 2 or more occasions atleast 12 weeks apart measured by standard ELISA .

Antiphospholipid antibody syndrome is present when one or more of the clinical and one or more of the laboratory criteria are present.

DIAGNOSIS

Patients suspected of having antiphospholipid syndrome should be tested using at least 2 antiphospholipid antibody assays. The must commonly performed are LAC and ACL.Testing for LAC utilizes in vitro coagulation assays include activated Partial Thromboplastin Time, diluted Russell's viper Venom Test, Kaolin Clotting Time, tissue thromboplastin inhibition test. The test for ACL is standardized ELISA.

LAC tests are reported as positive or negative, ACL as in terms of international units of GPL and MPL.

Creagh et al., (1991) investigated 66 women with first or second trimester fetal loss for the presence of lupus anticoagulant by routine anticoagulant tests, dilute Russell's viper venom time test, and for raised anticardiolipin antibodies by ELISA. Of 35 women with recurrent fetal loss, seven were positive for LAC, 6 had increased IgG anticardiolipin antibodies, while of 31 women with only one or two fetal losses, one had LAC, none had increased IgG ACL antibody. These were significant. From this study it is concluded that lupus anticoagulant and increased IgG anticardiolipin antibodies are independently associated with recurrent first or second trimester fetal losses and such cases should be investigated.

Another study by **Mac Lean et al.,** (1994) provides evidence of an association between lupus anticoagulant and raised anticardiolipin antibodies and early pregnancy loss. A prospective study to determine the prevalence of lupus anticoagulant and raised anticardiolipin antibodies in women with two or more miscarriages in first trimester was conducted. Two hundred and forty three women [113 had 2 miscarriages, and 130 had 3 or more miscarriages] were studied, of the 243 women tested, 16 (6.6%) were positive for LAC, 20 (8.2%) had elevated ACL, and 5 (2%) had both the antibodies. The most frequently positive tests were dilute Russell`s viper venom time and IgG for elevated anticardiolipin antibody.

A case control study conducted in Calcutta in 1996 by **Bhattacharya et al**., with 41 women with recurrent pregnancy wastages showed 19.5% of positivity for LAC. The tests used were APTT and KCT. This study also showed that the frequency of pregnancy wastage were more common in first and third trimester than second trimester.

Antiphospholipid antibodies can be detected in 1-5% of normal population without pregnancy loss. This is similar to the study by **Kutteh et al.,** (1996). In this study, prevalence of antiphospholipid antibodies in general population was 4% and in women with recurrent pregnancy loss was 17.3%.

A case control study of the association between recurrent pregnancy loss and LAC and ACL was conducted by **Gűllinnaz ALPER and colleagues** (1999). In this study 25 women with 2 or more consecutive unexplained spontaneous abortions and 15 women with one or more normal pregnancies without previous abortion were taken as cases and controls respectively. ELISA and APTT tests were performed. Mann-Whitney non parametric test was used for analysis. LA activity was detected in 5 (20%) of cases and none in controls. Increased ACL levels were observed in 8 of 25 (32%) cases and one of 15 controls (7%).

Detection of antiphospholipid antibodies must be considered in women with previous pregnancies complicated by unexplained fetal wastages.

Chakrabarti et al., (1999), in his case control Study, found the prevalence of antiphospholipid antibodies on 50 pregnant women of first or second trimester with history of two or more unexplained pregnancy losses and 30 pregnant women of same trimester without history of fetal loss. LA was detected in 9 (18%) cases, and ACL in 12 (24%) cases of study group. The control group was negative for any antibody. The prevalence of antibodies in study group was statistically significant.

Kumar et al., (2002), in his case control study at Hyderabad, found the incidence of antiphospholipid antibodies on 150 couples experiencing 3 or more recurrent pregnancy losses with similar number of matched controls. Using student's test, LAC activity was found positive in 11 (10.28%) women and the binding of ACL antibodies to antigen was observed in 40.24% (n=33) of women.

This study suggested the usefulness of screening for these antibodies as a mandatory routine for a successful pregnancy outcome.

Zolghadri et al., (2004) determined the role of antiphospholipid antibodies as an etiological factor in recurrent pregnancy failure, in his prospective case control study on 138 women with history recurrent pregnancy loss and 100 well matched controls. ACL antibody was measured by ELISA and LA by activated PTT.16 (11.6%) had positive ACL in cases, while 3 (3%) in controls were positive for ACL, P = 0.0157, OR= 4.24.LA was positive in

12(8.7%) cases, and 3 (3%) controls. P = 0.074, OR = 3.08.Overall 24 women (17.4%) were positive for one of the antibody, P = 0.0005, OR = 6.81. Four were positive for both antibodies.

This study emphasized the relationship between antiphospholipid syndrome and recurrent pregnancy failure.

Velayuthaprabhu et al., (2005) in a sequential Study, analyzed the prevalence of anticardiolipin antibody on 155 women with history of 3 or more recurrent spontaneous abortion in first trimester. ELISA test was performed and analyzed by two tailed t-test to know the significance of ACL which was detected in 40% of cases.

Ghosh et al., (2006) in his study on 155 women with recurrent miscarriage/late pregnancy loss found a positive relationship of 27.7% for antiphospholipid antibody syndrome and recurrent pregnancy loss. This was significant.

The significance of antiphospholipid antibodies in women with bad obstetric history was studied by **Mishra et al.,** (2007) in Mumbai. A prospective study on 120 women with bad obstetric history by ELISA for ACL, and anticoagulant tests for LAC was done. This study showed a significant result of 28.3% and 15% of positivity for ACL and LAC respectively.

Another case control study was done by **Indu Koul** and **colleagues** (2007) in Jammu to assess the association of antiphospholipid antibodies in early repeated abortions. For detecting LA, prothrombin time, APTT, KCT were used and for ACL, ELISA was used. Study population was 50 women without pregnancy loss and 50 patients with two or more previous pregnancy wastages in any trimester. Using Fisher test, statistical analysis was made. Among study group, 7 were positive for LAC, 5 were positive for ACL, P value for LAC = 0.012, for ACL = 0.056. This was significant. All the controls were negative for antibodies.

This study also reported the association with early onset preeclampsia, intrauterine growth restriction and placental abruption.

A prospective study by **Ajami et al.,** (2007) to detect the prevalence of IgG anticardiolipin antibody in recurrent pregnancy loss - Sari, concluded that the antibody is higher in second and third trimester of patients with recurrent fetal losses. 512 women in the age range of 18 -40 years (men 28.02 ± 5.66) were included. ELISA test was performed. ACL was detected in 57 (11.1%) cases (CI =2.7).

A retrospective study was conducted in Brazil, by **Spegiorin LCJF and colleagues** (2010) to assess the prevalence of ACL antibody in patients with repeated miscarriages. 52 women with history of two or more miscarriages were evaluated, in the age range of 17-42, [mean age 26.7 years],

ELISA test was performed. Fisher exact test was used for analysis. Abnormally high ACL antibody level was found in 55.7% of cases and LA in 2% of cases.

Recently a study was conducted by **Sarra Klai and colleagues** (2010) to assess the association of antiphospholipid antibodies in pregnancy related complications. 302 women with pregnancy complications and 100 women with past history of uncomplicated pregnancies were screened for LAC and ACL antibodies .Significant association was found between antibody positivity and recurrent pregnancy loss (OR = 16.87, 95% CI, 5.5 – 51.63, $P < 10^{-3}$) , intrauterine growth restriction (OR 3.9; 95% CI 1.08 -14.05, P = 0.04), Preeclampsia (OR = 15.31; 95% CI 1.25 – 16.42, P = 0.035).IgG was considered a risk factor for intrauterine growth restriction and recurrent pregnancy loss and LAC was associated with recurrent pregnancy loss alone.

The assay for ACL antibodies is more sensitive and assay for LAC is more specific.

Consensus guidelines recommend that at least 2 different phospholipid dependent assays should be used for screening and mixing studies with normal plasma should fail to correct the prolongation. Other coagulopathies should be excluded. The levels of LAC and ACL show spontaneous variation between pregnancy and non pregnancy, and even in the same pregnancy. Hence a single

abnormal test must be repeated after at least 8 weeks before it is considered clinically significant. Although majority of patients with antiphospholipid syndrome have positive ACL and LAC, approximately 10-16% are positive for LAC and negative for ACL, 25% are positive for ACL and negative for LAC (17).

The **American College of Obstetricians and Gynecologists (ACOG)** guideline of Management of Recurrent early pregnancy loss (2001) reached the following conclusion:

Women with recurrent pregnancy loss should be tested for lupus anticoagulant and anticardiolipin antibodies using standard assays. If test results are positive for the same antibody on two consecutive occasions 6 – 8 weeks apart, the patients should be treated with heparin and low- dose aspirin during her next pregnancy attempt. Mononuclear cell (leukocyte) immunization and intravenous immunoglobulin (IVIG) are not effective in preventing recurrent pregnancy loss.

The **Royal College of Obstetricians and Gynaecologists** Guidelines on Management of Recurrent Miscarriage (2001) are consistent with **ACOG** Guidelines. **RCOG** recommends, screening tests for antiphospholipid antibodies (both the lupus anticoagulant and anticardiolipin antibodies)

performed on two separate occasions at least six weeks apart. Discordant results should prompt the performance of a third test.

Regarding management, those women with persistently positive tests for antiphospholipid antibodies are offered treatment with low dose aspirin together with low dose heparin during pregnancy (also the subject of on – going research).

TREATMENT

Treatment modalities are modified according to the type of antiphospholipid syndrome. Definite or Classic APS includes patients with LAC / medium to high titers of IgG/IgM ACL and fetal death, recurrent preembryonic or embryonic pregnancy loss, neonatal death after delivery for severe preeclampsia or fetal distress or thrombosis.

Treatment options have evolved considerably. Early enthusiasm for glucocorticoids waned when a small, randomized trial found heparin administered to pregnant women to be as effective as prednisone (18). Recently, two prospective trials showed that heparin plus low-dose aspirin is more effective than aspirin alone for achieving live births among women with antiphospholipid antibodies and predominantly preembryonic and embryonic

pregnancy loss. A third prospective trial of women who were positive for antiphospholipid antibodies and has repeated pregnancy loss but no history of thrombosis or systemic lupus erythematosus found similar rates of live birth (approximately 80 percent) with the use of either low-dose aspirin or placebo, suggesting that treatment may be unnecessary in some women (19).

Concern about patient selection notwithstanding, most experts recognize the antiphospholipid syndrome as a proven, treatable cause of recurrent pregnancy loss. Heparin and low dose aspirin treatment should be initiated after the identification of a viable pregnancy documented by ultrasound. The recommended dose is Injection Heparin 15000-20000 units/day subcutaneously in divided doses with low dose aspirin 80 mg/day (20). This should be combined with calcium and vitamin D supplementation to be continued postpartum (6 weeks). They can be switched over to warfarin postpartum.

Low dose aspirin may improve pregnancy outcome by irreversibly blocking the cyclo-oxygenase in platelets, thereby inhibiting thromboxane synthesis. Heparin may act by binding to phospholipid antibodies, thereby protecting the trophoblast phospholipids.

Two types of immunotherapy have been explored; injections of paternal leukocytes (paternal white blood cell immunization or paternal cell alloimmunization) and the use of intravenous immunoglobulin (IVIG).

Recent meta-analyses of randomized controlled trials of immunotherapy for recurrent miscarriage concluded that IVIG and paternal leukocyte injections provided no significant beneficial effect over placebo in preventing further miscarriages (Porter et al., 2006). The investigators also found no significant benefit for other immunological treatment that has been used for recurrent miscarriage: third party donor cell immunization and trophoblast membrane infusion.

An **American Society for Reproductive Medicine (2004) Committee** Opinion concluded that, IVIG as treatment of recurrent pregnancy loss should be evaluated in patients who are informed, consenting participants in an institutional review board approved randomized clinical trial. For the management of recurrent spontaneous pregnancy loss IVIG is an experimental treatment.

AIM OF STUDY

AIM OF STUDY

The aim of study is to screen for the presence of lupus anticoagulant antibodies and anticardiolipin antibodies in patients with unexplained recurrent pregnancy loss to evaluate the association.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study Setup

This study on the screening for lupus anticoagulant and anticardiolipin antibody in women with unexplained recurrent pregnancy loss was a prospective analytical study undertaken at Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for women and children, Chennai during December 2009- November 2010.

Inclusion Criteria

Fifty antenatal mothers with previous history of two or more unexplained recurrent pregnancy losses were taken up for the study.

Controls

The controls were 50 healthy pregnant women matched for age and gestational age with no history of previous abortions and have borne one or more live children.

Exclusion criteria

The mothers with history of following conditions were excluded from the study. The conditions were,

- Diabetes
- Rh incompatibility
- Endocrine causes
- Genetic causes
- Coagulation disorders
- Sexually transmitted diseases.
- Anemia
- Hypertension
- Uterine anomalies
- Autoimmune disorders.

Study design

For the selected number of patients, a careful detailed history was taken with special attention to previous obstetric history details regarding the previous pregnancy loss like abortion, intra uterine fetal death were recorded. Careful systemic and obstetric examinations were done.

Routine and special investigations were done, these include

- Urine :Albumin

  Sugar

  Deposits

  Culture and sensitivity

- Haemogram and Packed cell volume
- Platelet count
- Blood grouping and Rh typing
- Bleeding time and clotting time
- Blood urea
- Blood sugar
- Serum creatinine
- Serum uric acid
- Plasma Fibrinogen
- VDRL
- HIV with consent
- Ultra Sonogram
- Endocrinologist opinion
- Genetic opinion
- Diabetologist opinion

TEST FOR LUPUS ANTICOAGULANT ANTIBODY

Procedure

Blood samples for coagulation studies were obtained by venepuncture, collected into glass tubes containing 3.8% trisodium citrate as the anti-coagulant in the ratio 1 part anticoagulant to 9 parts of blood. For each study sample, one control sample was taken. Platelet poor plasma was obtained by centrifuging blood for 15 minutes and used for LAC assays. The tests were done at Department of Biochemistry and Department of Rheumatology, Government General Hospital, chennai.

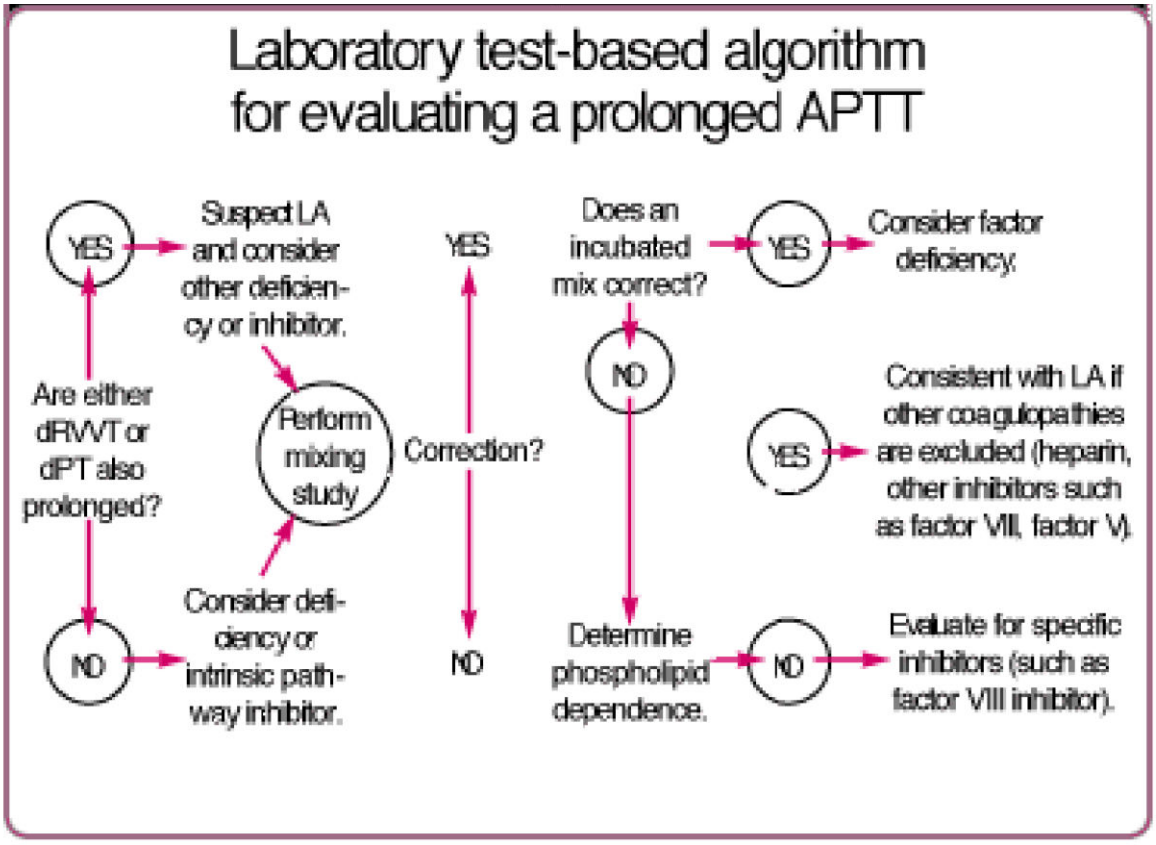
The coagulation tests done for LAC were,

1) Activated Partial Thromboplastin Time (APTT)
2) diluted Russell Viper Venom Time (dRVVT)

Activated Partial Thromboplastin Time

Principle

The test measures the clotting time of test plasma after the addition of APTT reagent, then allowing activation time, followed by the addition of Calcium Chloride.


Laboratory test-based algorithm for evaluating a prolonged APTT
YES
Suspect LA and consider other deficiency or inhibitor.
Are either dRVVT or dPT also prolonged?
Perform mixing study
Correction?
YES
NO
Consider deficiency or intrinsic pathway inhibitor.
NO
Does an incubated mix correct?
YES
Consider factor deficiency.
NO
YES
Consistent with LA if other coagulopathies are excluded (heparin, other inhibitors such as factor VIII, factor V).
Determine phospholipid dependence.
NO
Evaluate for specific inhibitors (such as factor VIII inhibitor).

Deficiencies of approximately 40% and lower of factors VIII, IX, XI and XII will result in a prolonged APTT. Heparin in the presence of adequate amounts of AT-III will also result in a prolonged APTT. The APTT reagent contains a near colloidal particle activator (magnesium-aluminium silicate) for optimum sensitivity to factor deficiencies and to heparin. The reagent also contains a chloroform extract of rabbit brain with buffer and stabilizers (21).

Materials used

1. Platelet poor plasma – from both test and control cases

2. Calcium chloride 0.025M

3. APTT reagent

4. Precision pipette 0.1ml

5. Suitable timer

6. Water bath at constant temperature of 37˚C

Technique

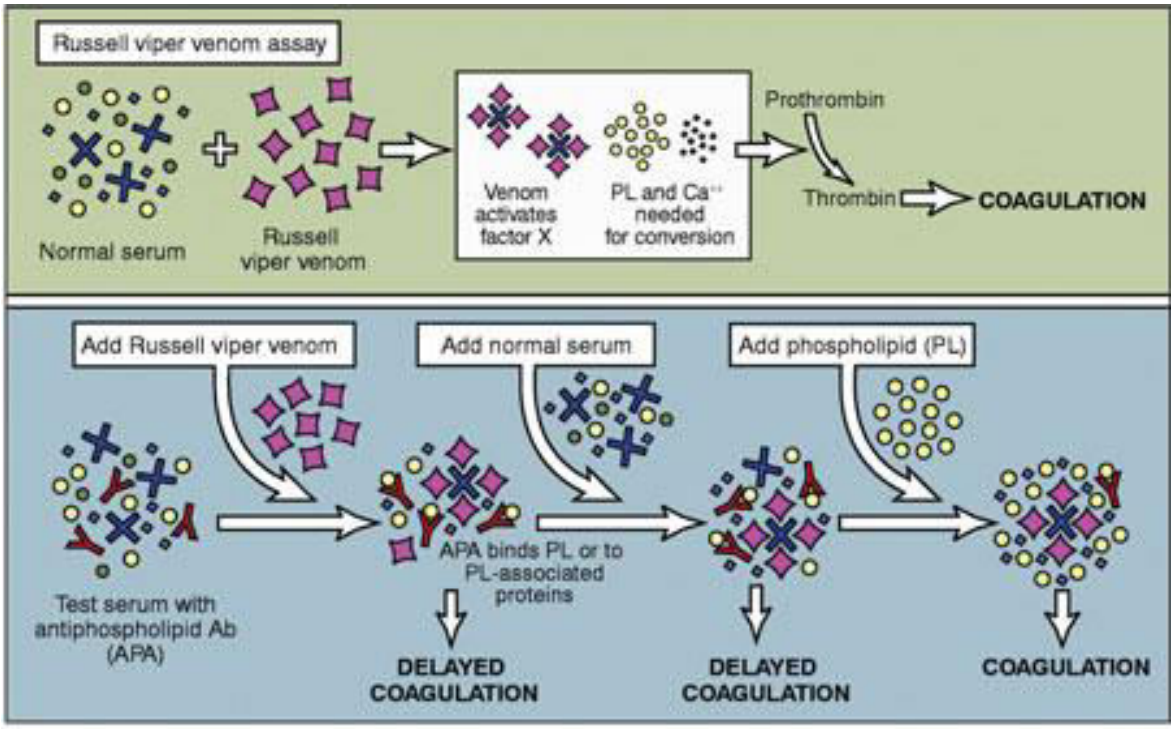
The test plasma is prewarmed to 37˚C for 2 minutes. The APTT reagent is also prewarmed to 37˚C. 0.1 ml of reagent is forcibly added to 0.1ml of test plasma and this is incubated at 37˚C for exactly 5 minutes. To this, 0.1ml of

0.025 M Calcium Chloride (prewarmed to 37˚C) is added and the time for clot formation is noted. The same procedure is repeated for the control plasma. The normal value is between 30-50 seconds, in LAC positive patients it is not correctable by 1:1 mixing with normal plasma. LAC is present if the difference in clotting times between the patient and control plasma is more than 10 seconds.

Dilute Russell`s Viper Venom Time

Principle

Russell's viper venom directly activates factor X in the presence of phospholipids and Calcium ions, bypassing factor VII of extrinsic pathway and the contact and antihaemophilic factors of the intrinsic pathway. In normal plasma in the absence of lupus anticoagulants, factor X is directly activated by Russell's viper venom, which in the presence of phospholipid and calcium ions leads to clot formation. In patients with LAC, auto antibodies bind the epitopes of reagent phospholipids thereby preventing the activation of prothrombinase complex. This results in a prolongation of clotting time.



Antiphospholipid antibody determination of LA by the dRVVT.

Materials used

1) Platelet poor plasma-from both test and control cases
2) LA screen and LA confirm reagents-lyophilized preparations containing Russell's viper venom enriched with phospholipid at different concentrations, with 0.01% thiomersal as preservative.
3) Calcium chloride 0.025M
4) 0.1ml precision pipettes
5) Stopwatch
6) Glass test tubes
7) Water bath

Technique

All reagents should be brought to room temperature before prewarming at 37˚C for testing purpose .0.1ml of LA screen reagent is added to 0.1ml of platelet poor plasma, contents mixed and incubated at 37˚C for 3 minutes. To this, 0.1ml of calcium chloride is added (prewarmed at 37˚C for 10 minutes), and the time for clot formation is noted. If this screen time is less than 35 seconds, it indicates absence of LA and there is no need to perform the confirmatory test. If the screen time is more than 35 seconds, the same procedure is repeated with LA confirm reagent (this reagent incorporates

additional phospholipids to neutralize LA, thereby achieving a lower clotting time, thus proving the phospholipid dependence of the auto antibodies) (21).

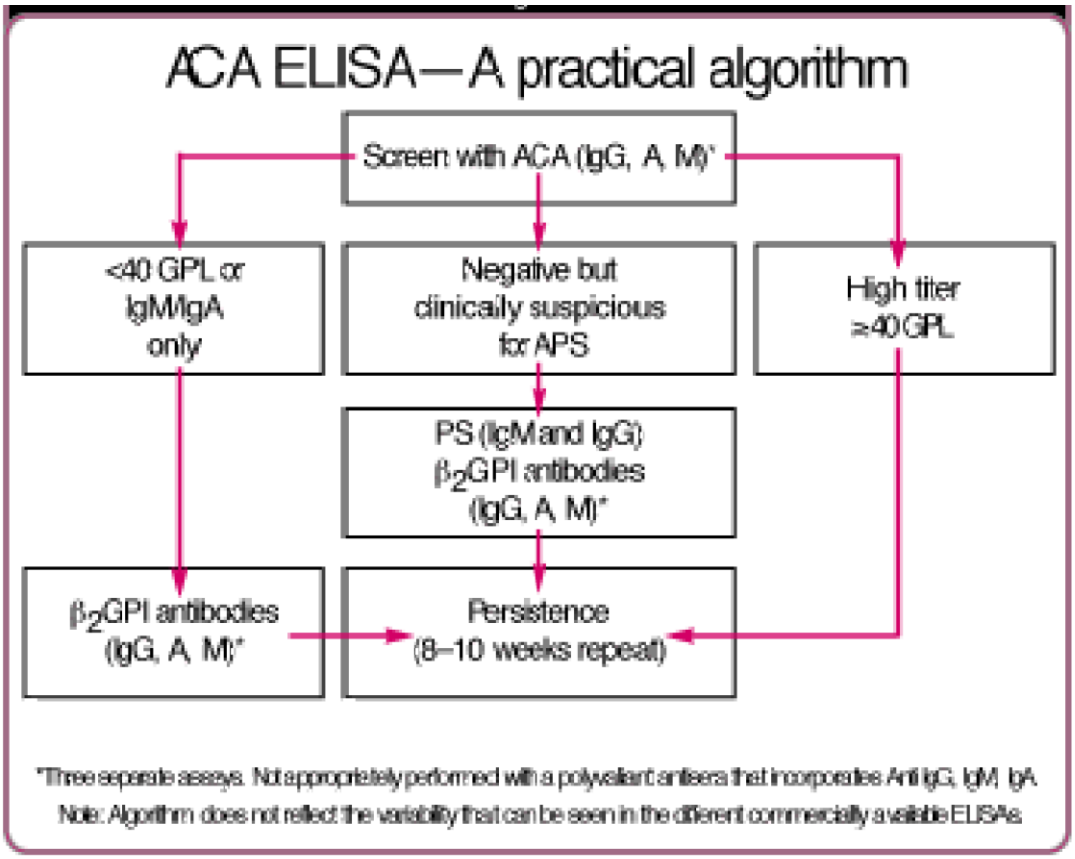
TEST FOR ANTICARDIOLIPIN ANTIBODY-ELISA

Principle

Enzyme immunoassay measures the enzyme labeled antigen, hapten, or antibody. It is of 2 types. (Homogenous and heterogeneous). ELISA is the heterogeneous EIA, requires the separation of free and bound fractions either by centrifugation or by absorption on solid surfaces and washings. The reagents are added sequentially.

Materials used

1. Serum from test and control cases
2. Microtitre plates
3. Cardiolipin
4. Blocking buffer
5. Horse raddish peroxidase
6. Hydrogen peroxide
7. Stop solution.


ACA ELISA—A practical algorithm
Screen with ACA (IgG, A, M)*
<40 GPL or IgM/IgA only
Negative but clinically suspicious for APS
High titer ≥40 GPL
PS (IgM and IgG) β2GPI antibodies (IgG, A, M)*
β2GPI antibodies (IgG, A, M)*
Persistence (8–10 weeks repeat)
*Three separate assays. Not appropriately performed with a polyvalent antisera that incorporates Anti IgG, IgM, IgA
Note: Algorithm does not reflect the variability that can be seen in the different commercially available ELISAs

Technique

Serum is the recommended sample for ACL evaluation. Anticardiolipin antibody estimation is done by ELISA method (**Harris et al**). Individual 96 well micro titer plates are coated with 30 ml of cardiolipin at a concentration of 45 mg per ml and blocked with 200 ml of blocking buffer containing 10% fetal calf serum for 2 hours. 50ml of test samples in 1:150 dilutions with PBS are added to react. After washing, equal amounts of purified Horse raddish peroxidase is added and allowed to react for 3 hours at 4˚C. Again plates are washed. H_2O_2 is added at 56˚C until the blue color is obtained, then stop solution is added and read at 405nm. The result is interpreted in GPL units or MPL units (<10-negative, 10-19-Borderline, 20-80 positive, >80 – high positive) (22).

DATA ANALYSIS

DATA ANALYSIS

The present study of screening for the presence of lupus anticoagulant and anticardiolipin antibodies in recurrent pregnancy loss was carried out on 50 pregnant women with previous two or more pregnancy wastages. The tests were also performed on 50 controls. The various observations from this study were analyzed using chi-square test wherever necessary and the results were tabulated here.

Table-1

Age Distribution of all cases

Age in Yrs	No. of Patients	%
≤ 20	3	6%
21-24	18	36%
25-29	19	38%
≥30	10	20%
	50	100%

This table shows the age distribution of cases. The maximum number of patients fell into 25 - 29 years (38%) age group.36 % belonged to 21-24 years, 20% to > 30 years, and six percentage to < 20 years age group.

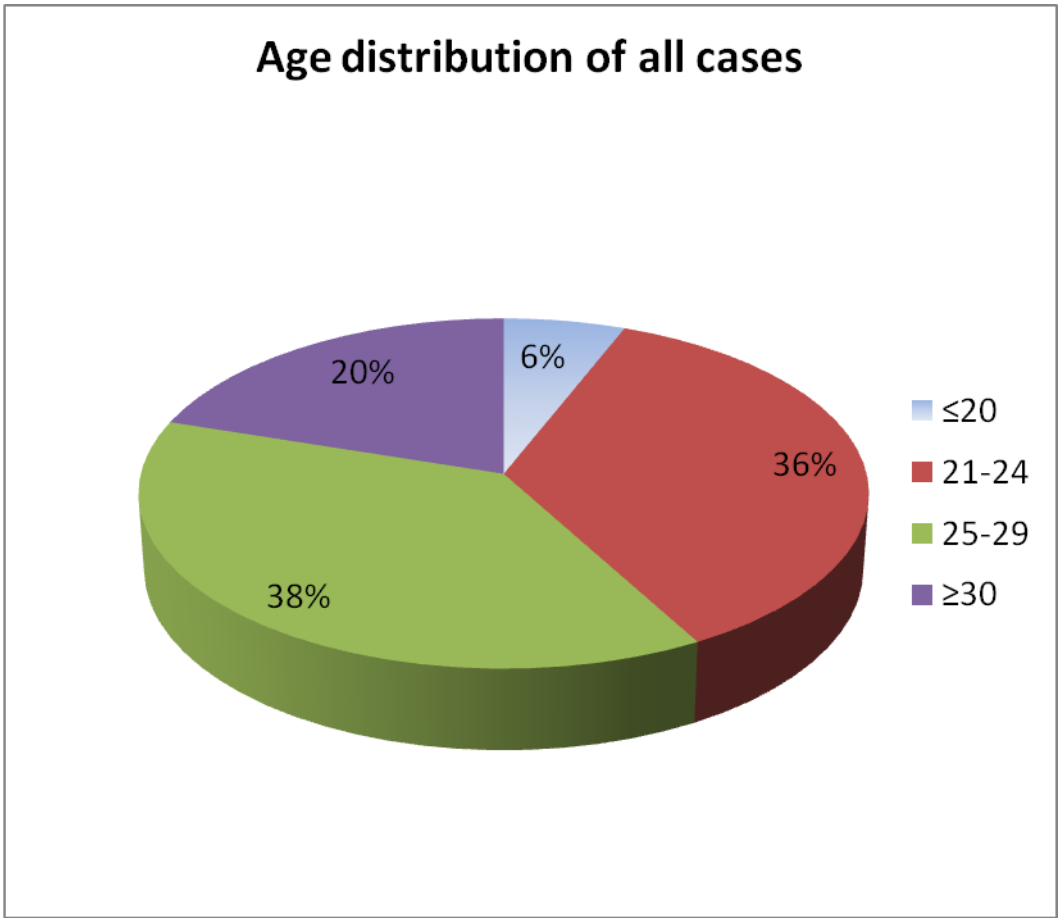

Age distribution of all cases
6%
36%
38%
20%
≤20
21-24
25-29
≥30

Table-2

Comparison of age distribution of cases and controls

Age in Years	Cases	Controls
≤20	3	3
21-24	18	16
25-29	19	21
≥30	10	10

Mean	25.92	25.98
SD	4.125	4.138

P=0.975

This table shows that the age distribution of cases and controls with the maximum number of cases falling in the age group 25-29 years. The cases and controls are comparable with age.

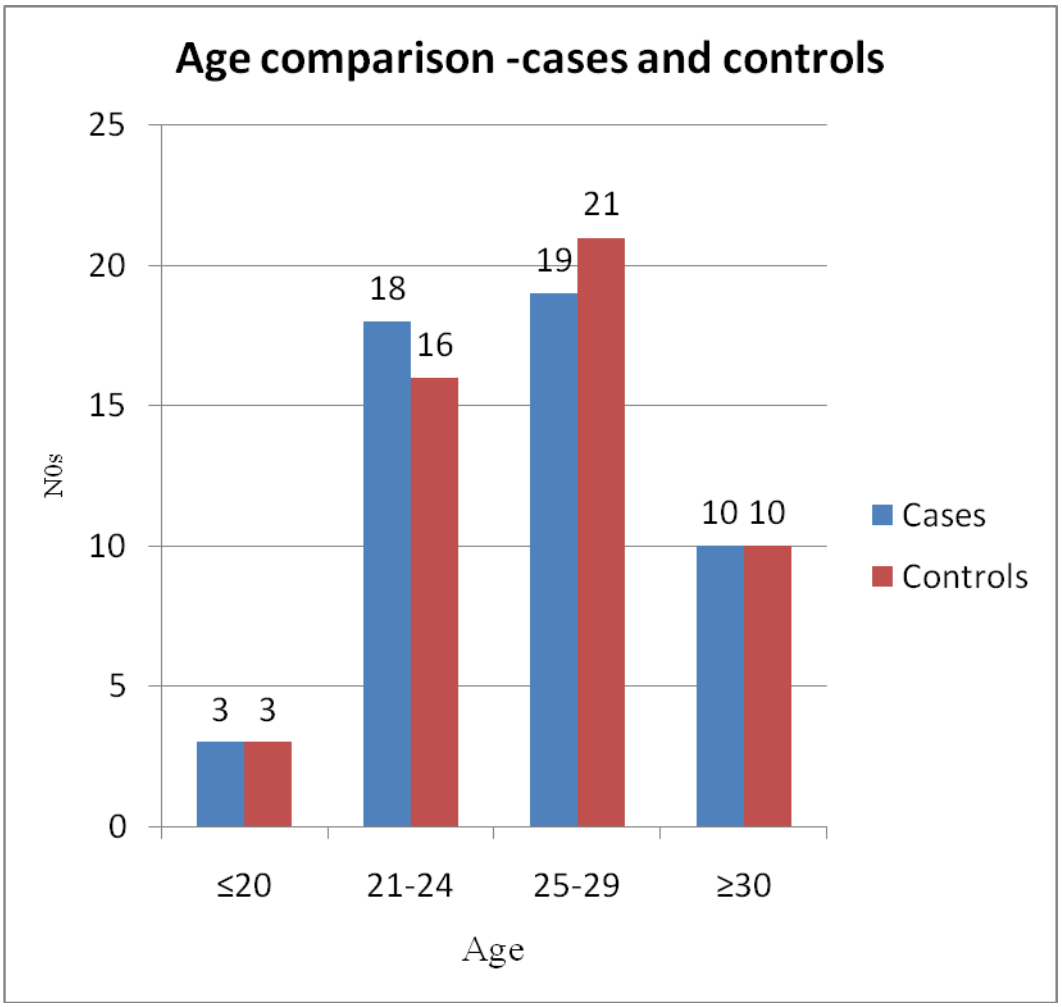

Age comparison -cases and controls
25
20
15
10
5
0
Nos
3
3
18
16
19
21
10
10
Cases
Controls
≤20
21-24
25-29
≥30
Age

Table-3

Conception distribution of cases

Gravida	No .of cases	%
3	13	26%
4	25	50%
5	12	24%

This table shows that among the cases, maximum number were Gravida IV (50%), 26%were Gravida III, 24%were Gravida V.

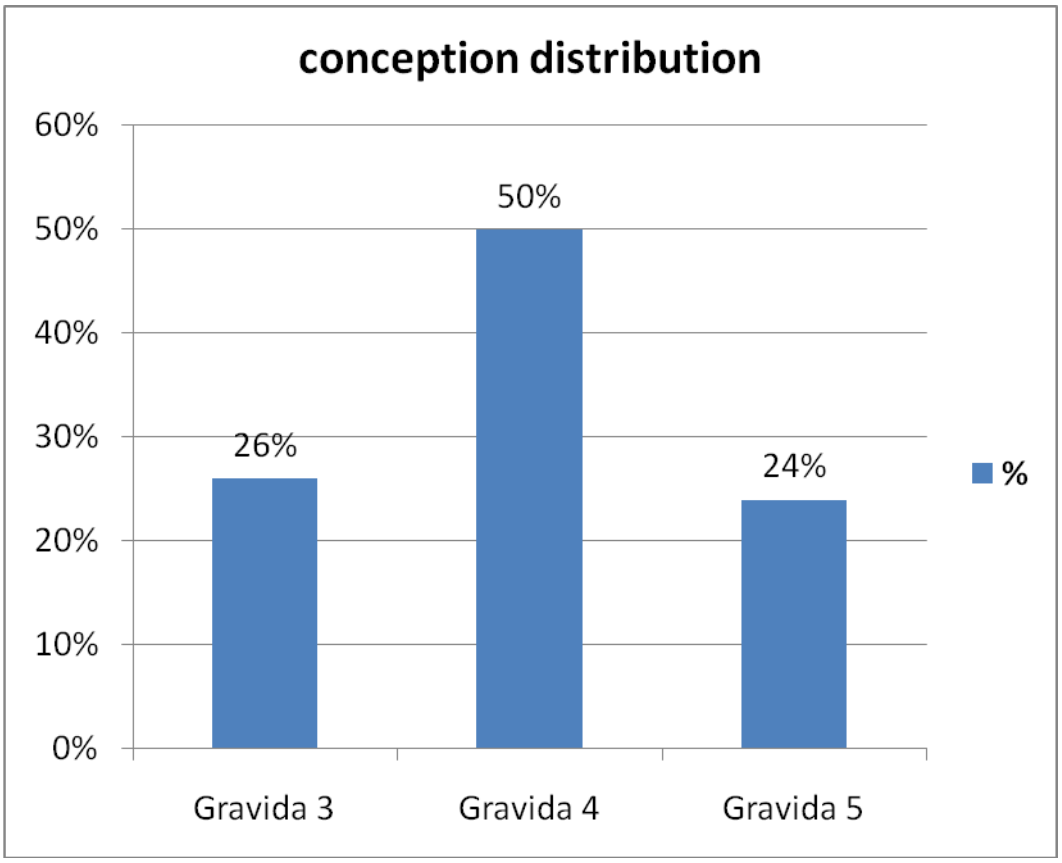

conception distribution
60%
50%
40%
30%
20%
10%
0%
26%
50%
24%
Gravida 3
Gravida 4
Gravida 5
%

Table-4

Comparison of Gestational age of cases and controls

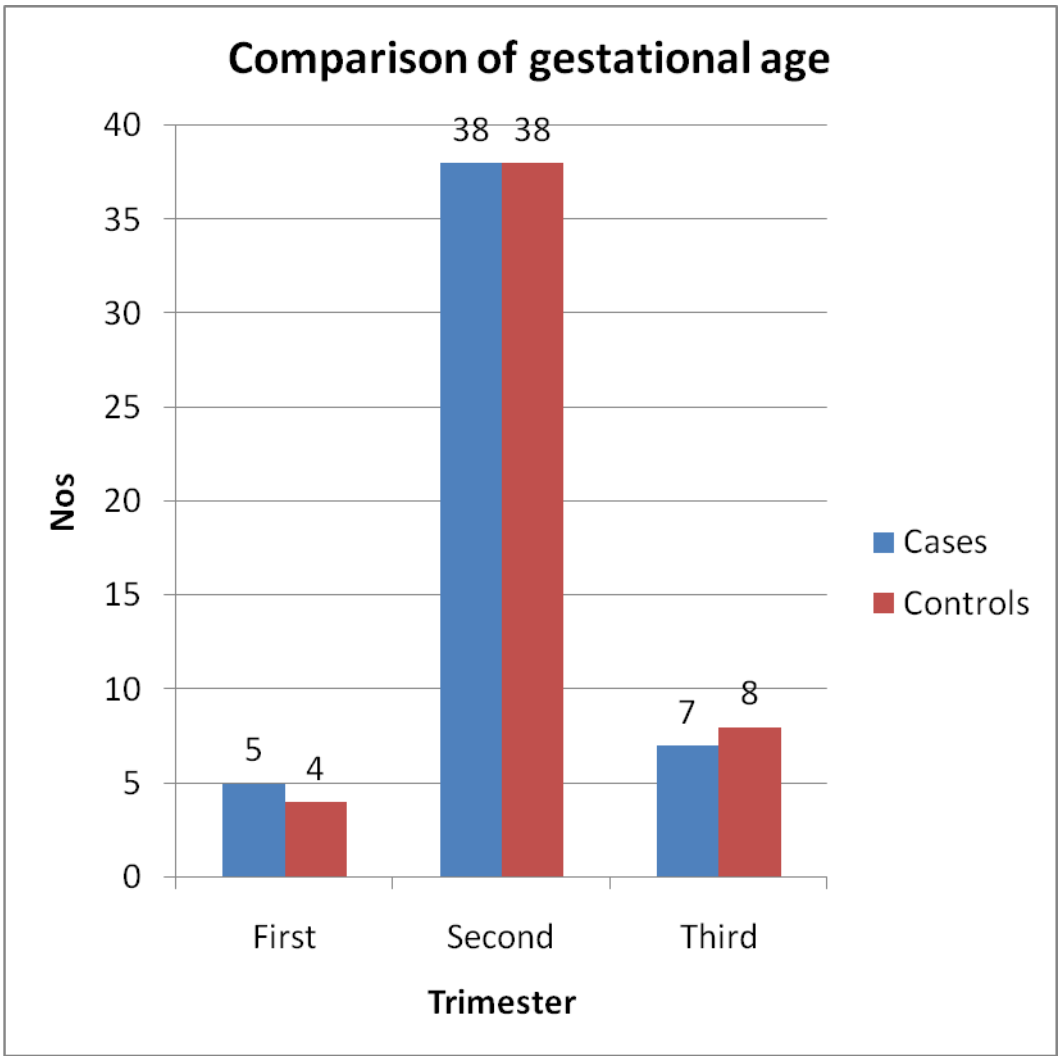
Trimester	Cases	Controls
First	5	4
Second	38	38
Third	7	8

Mean	22.02	22.48
SD	6.022	6.072

P=0.969

This table shows that maximum number of patients were in second trimester.

S.No	Age	Parity	G.A	LAC tests	ACL tests	Pregnan



				APTT		dRVVT		IgM	IgG	
				Study	Control	Study	Control			
1	24	G5P2L0A2	14	54	35	52	36	Absent	Present	A
2	26	G5P0L0A4	18	60	32	58	33	Absent	Present	A
3	29	G3P2L0A0	28	52	32	55	36	Absent	Present	FD
4	33	G4P0L0A3	20	58	40	55	34	Absent	Present	A
5	30	G5P2L1A2	17	69	40	58	40	Absent	Present	LB
6	21	G4P0L0A3	16	34	32	35	31	Absent	Present	LB
7	28	G3P0L0A2	26	36	34	32	30	Absent	Present	LB
8	30	G3P2L2	28	56	36	52	37	Absent	Absent	LB
9	28	G2P1L1	32	54	34	52	38	Absent	Absent	LB
10	27	G2P1L1	12	36	37	35	34	Absent	Present	LB

Table -5

Antibodies positive cases and controls

G.A=gestational age.

Among cases, 5 were positive for LAC, 7 were positive for ACL.

Among controls, 2 were positive for LAC, 1 was positive for ACL.

Table-6

Lupus anticoagulant antibodies positive patients (APTT and dRVVT test)

	No. of Test positives	No. of Test negatives	Total
Cases	5	45	50
Controls	2	48	50
Total	7	93	100

Sensitivity=71.42%

Specificity=51.61%

Positive Predictive Value=10%

Negative Predictive Value=96%

False Negative=28.57%

False Positive=48.38%

The same 5 patients were positive for APTT and dRVVT test.

Table-7

Anticardiolipin antibodies positive patients (ACL IgG assay)

	No. of Test positives	No. of Test negatives	Total
Cases	7	43	50
Controls	1	49	50
Total	8	92	100

Sensitivity=87.5%

Specificity=53.26%

Positive Predictive Value =14%

Negative Predictive Value =98%

False Negative =12.5%

False Positive =46.73%

Table-8

Comparison of ACL test with LAC tests

Tests	Patients positive for ACL	Patients negative for ACL	Total
Patients positive for LAC	5	2	7
Patients negative for LAC	3	90	93
Total	8	92	100

Sensitivity=62.5%

Specificity=97.82%

Positive Predictive Value=71.42%

Negative Predictive Value=96.77%

Table-9

Previous Pregnancy Outcome of all Cases

Type of Loss	Numbers	%
Abortion	111	74.49%
Fetal death	32	21.47%
Live birth	6	4.02%
	149	

Among the total number of fetal losses 74.49% (n=111) were abortions, 21.47% were fetal deaths (n=32).

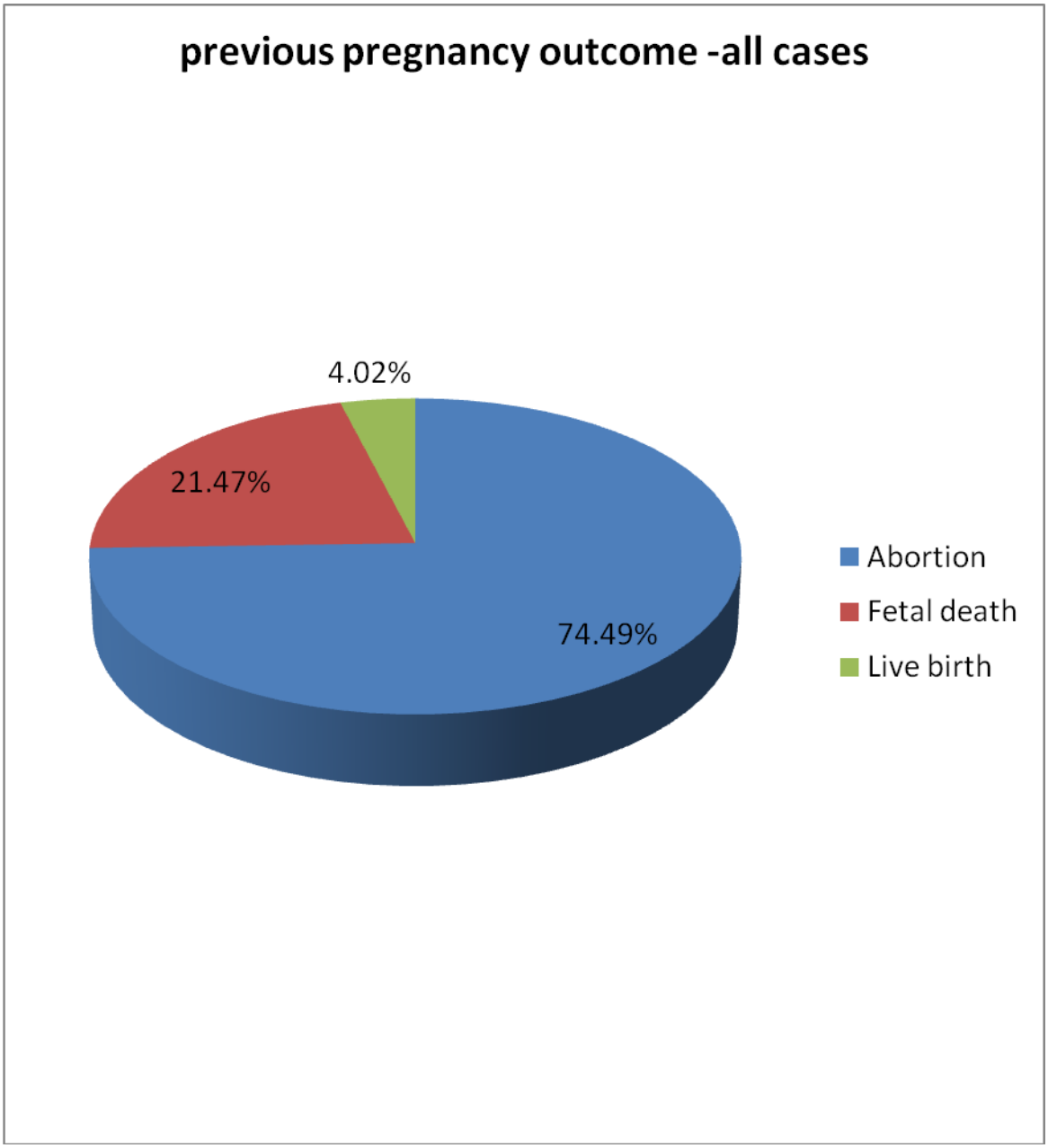

previous pregnancy outcome -all cases
4.02%
21.47%
74.49%
Abortion
Fetal death
Live birth

Table-10

Previous abortions of all cases.

No .of abortions	No .of cases	%
1	5	10.2%
2	27	55.1%
3	16	32.6%
4	1	2.0%

This table shows 55.1% of cases had two abortions and 32.6% had three abortions.

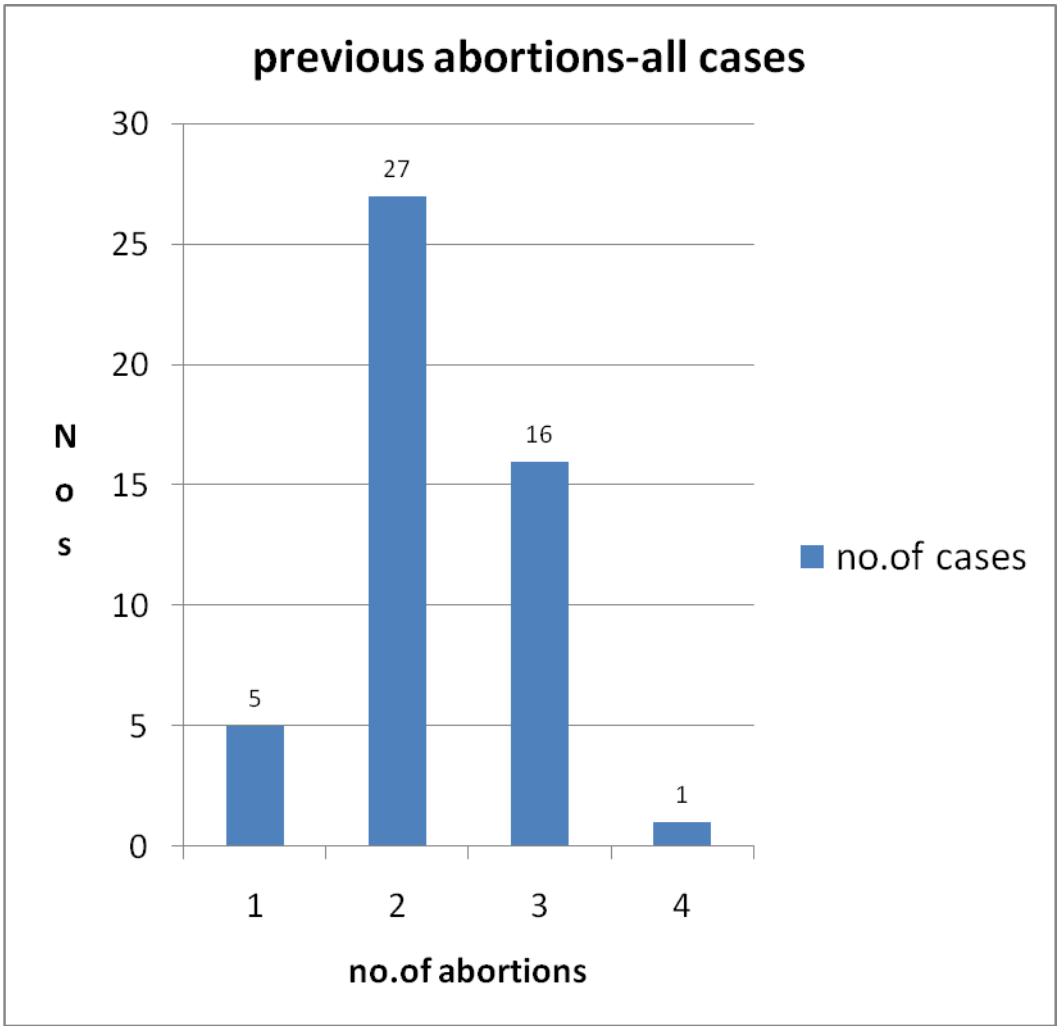

previous abortions-all cases
30
25
20
15
10
5
0
Nos
27
16
5
1
1
2
3
4
no.of abortions
no.of cases

Table-11

Present Pregnancy outcome in cases and controls

Outcome	Cases	Controls
Fetal loss	4	0
Live birth	46	50

P=0.041

Among cases, 46 patients gave birth to live children, 3 had abortions and one had fetal death. All the controls gave birth to live children

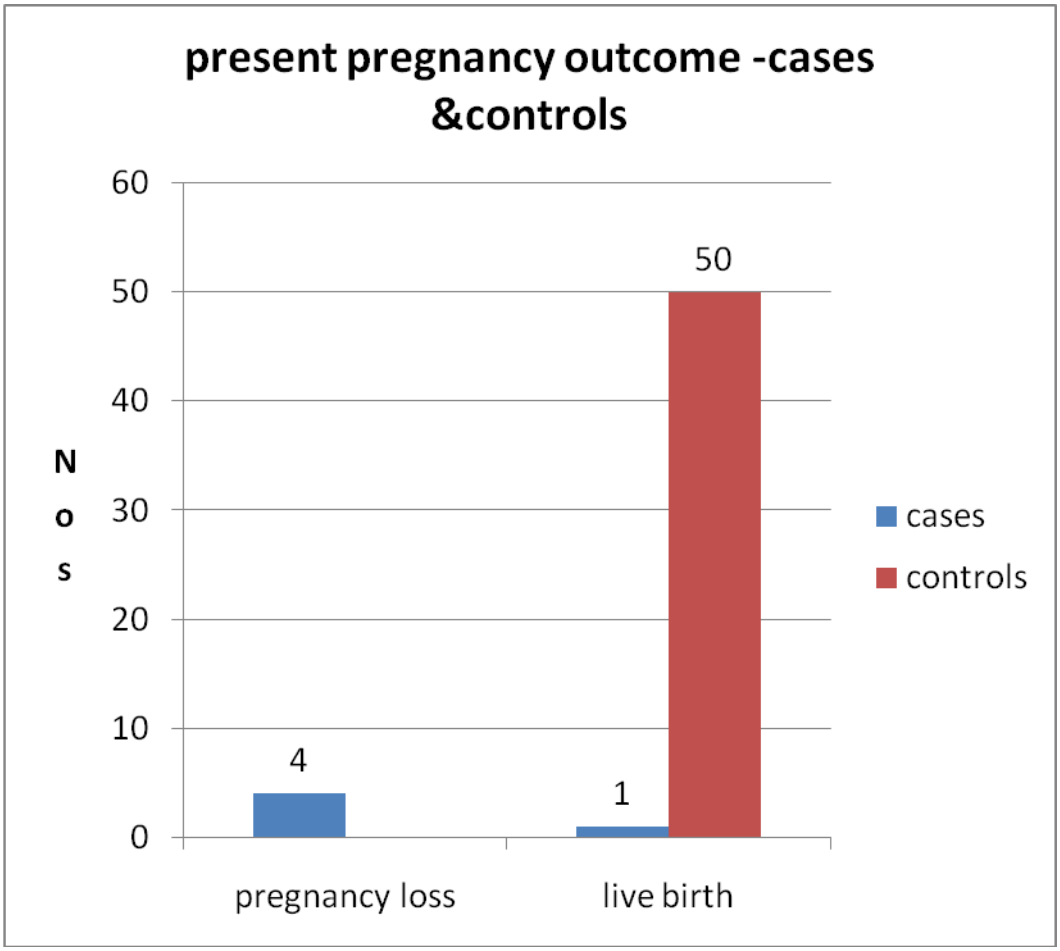

present pregnancy outcome -cases &controls
60
50
40
30
20
10
0
Nos
50
4
1
pregnancy loss
live birth
cases
controls

Table-12

Present pregnancy outcome in LAC positive cases

Outcome	No. of cases	%.
Abortion	3	60%
Fetal death	1	20%
Live birth	1	20%

Among 5 LAC positive cases 60% had abortions, 20% had fetal deaths and 20%delivered live children.

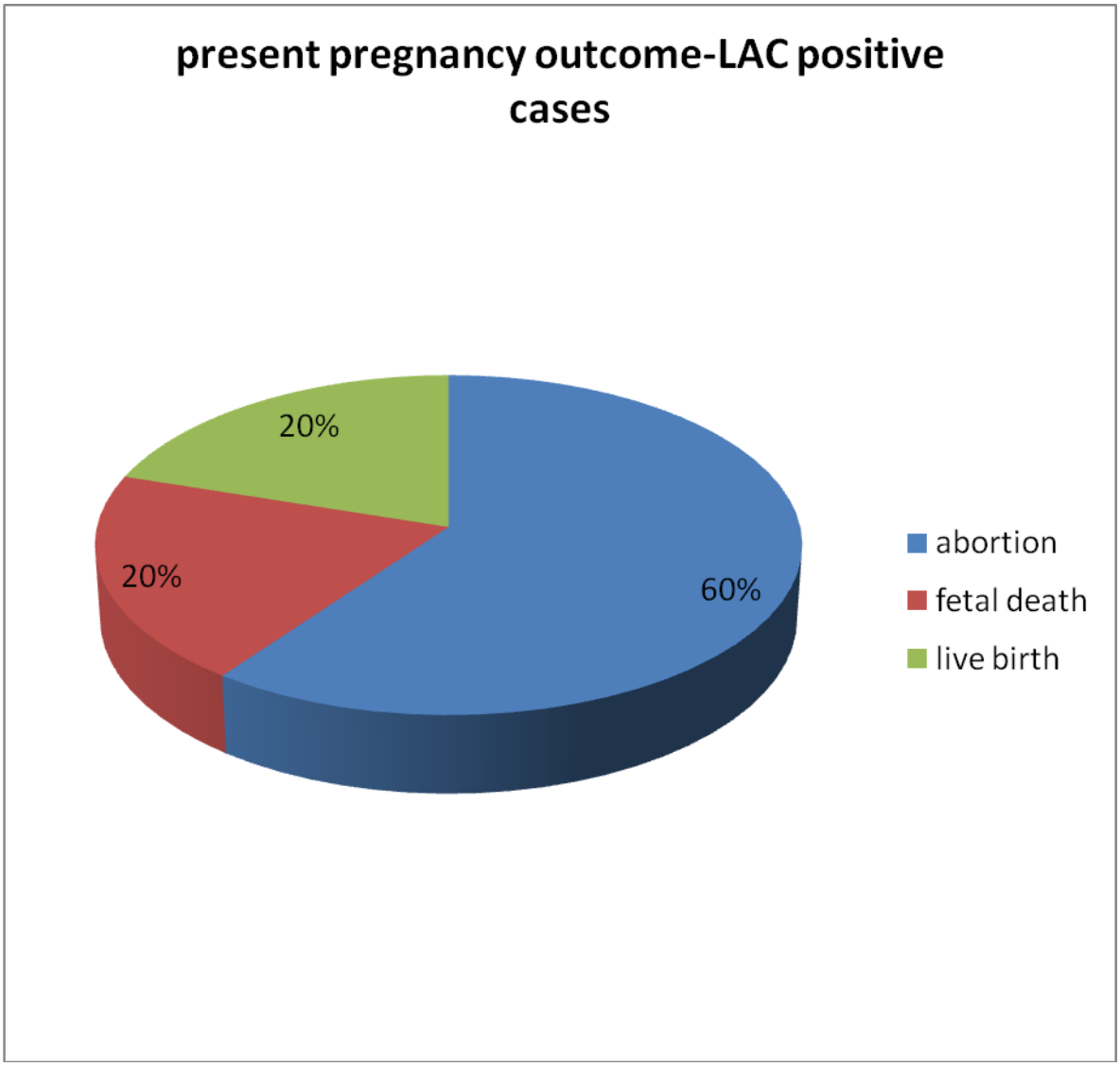

present pregnancy outcome-LAC positive cases
20%
20%
60%
abortion
fetal death
live birth

Table-13

Present pregnancy outcome in ACL positive cases

Outcome	No. of cases	%.
Abortion	3	42.85%
Fetal death	1	14.28%
Live birth	3	42.85%

Among 7 LAC positive cases 42.85% had abortions, 14.28% had fetal deaths and 42.85%delivered live children.

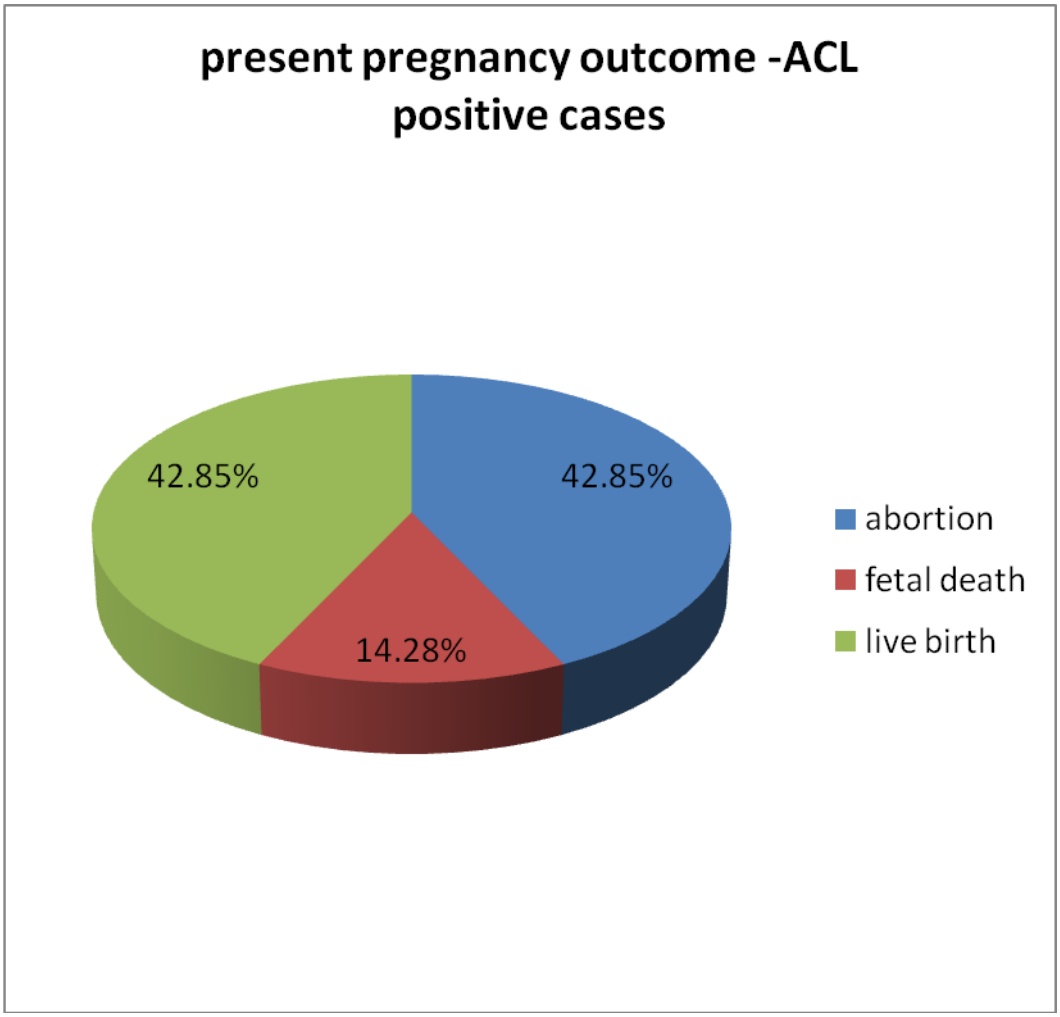

present pregnancy outcome -ACL positive cases
42.85%
42.85%
14.28%
abortion
fetal death
live birth

Table-14

Gestational age and present pregnancy loss

Gestational age	No. of losses	%
First trimester	0	0
Second trimester	3	42.8%
Third trimester	1	14.2%

This table shows 42.8% of pregnancy losses were in second trimester, and 14.2% were in third trimester.

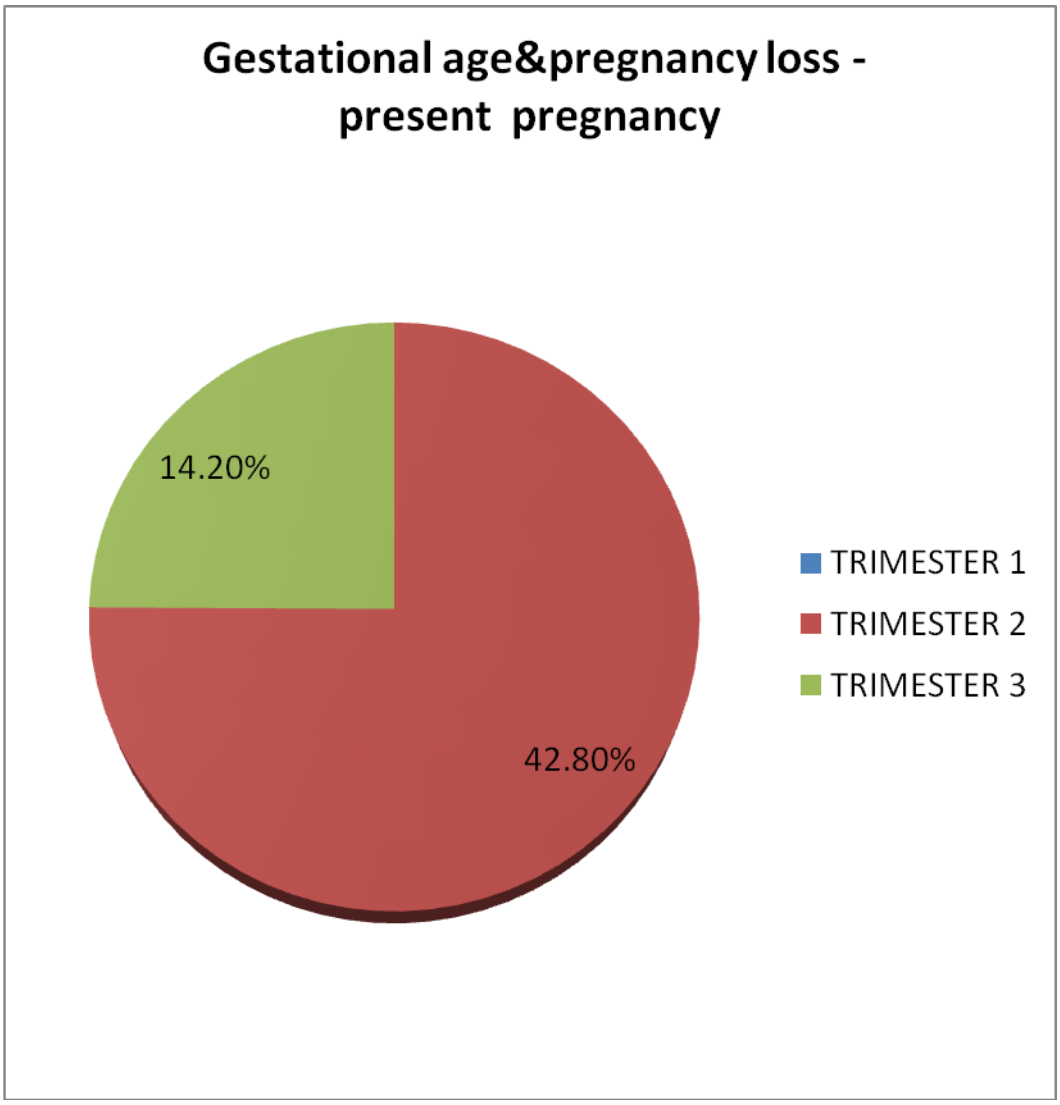

Gestational age&pregnancy loss - present pregnancy
14.20%
42.80%
TRIMESTER 1
TRIMESTER 2
TRIMESTER 3

Table-15

Previous &Present pregnancy outcome in APS cases

Pregnancy outcome	Previous pregnancy	%	Present pregnancy	%
Abortion	16	72.72%	3	42.85%
Fetal death	5	22.72%	1	14.28%
Live birth	1	4.5%	3	42.85%

P=<0.05

APS-antiphospholipid syndrome.

Table-16

Obstetric complications in previous pregnancy.

Complications	All cases	%	Antibody positive cases	%	P value
Abortions	111	74.49%	16	72.72%	0.00
Preeclampsia	35	23.48%	11	50%	0.00
IUGR	5	3%	2	9%	0.005
abruption	5	3%	2	9%	0.005
IUFD	32	21.47%	5	22.72%	0.00
PTL	2	1.34%	1	4.54%	0.05

IUGR-intrauterine growth restriction

IUFD-intrauterine fetal death

PTL-preterm labour

Table-17

Obstetric complications in present pregnancy.

Complications	No	%
Abortions	3	60%
Preeclampsia	2	40%
IUGR	1	20%
abruption	0	0%
IUFD	1	20%
PTL	1	20%

In the present pregnancy the occurrence of preeclampsia was found to be 40% and none reported to have abruption.

DISCUSSION

DISCUSSION

Recurrent pregnancy loss is defined as three or more consecutive losses which occurs approximately one in three hundred pregnancies (23). Clinical investigations may be initiated after two consecutive spontaneous losses, because in patients with recurrent pregnancy loss, the risk of subsequent pregnancy loss is 24%after two losses, 30%after three losses, and 40-50%after four losses (24).

Of the 100 patients who were screened for lupus anticoagulant antibodies using APTT and dRVVT tests, and anticardiolipin antibodies using ELISA, at Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital, Chennai, among patients with recurrent pregnancy loss, five cases were positive for lupus anticoagulant antibody, seven cases were positive for anticardiolipin antibody. Using APTT and dRVVT tests for detecting lupus anticoagulant antibody, the same five cases were positive for both the tests. All the patients were negative for test for anticardiolipin with IgM assay. Among the control patients (without pregnancy loss) two had positive results for lupus anticoagulant and one had positive result for anticardiolipin antibody.

Table-1&2 shows that ,majority of patients were in the age group of 25-29 years [cases – 38%, controls 42%]. The mean age for patients with recurrent pregnancy loss was 25.92 ± 4.12, and for patients without recurrent pregnancy loss was 25.98 ± 4.13.

P = 0.975, thus the age distribution between the cases and controls were comparable.

This is similar to the study conducted at Brazil by **Ligia** et al (2010), where the mean age of the cases were 26.7 years.

Table-3 shows the conception distribution as follows: Among the cases, majority were gravida IV (50%), 26% were gravida III, 24% were gravida V. In control group, 88% were gravida II, 10% were gravida III and two percentages were gravida IV.

As shown in table 4, the study and control groups were comparable in gestational age at which the tests were done. Among the cases, 5 (10%) were in first trimester, 38 (76%) were in second trimester, 7 (14%) were in third trimester. In the control group, 4 (8%) were in first trimester, 38(76%) were in second trimester, 8(16%) were in third trimester .p = 0.969.

Table-6 shows that seven patients showed prolongation of APTT and dRVVT tests, of which 5 were cases, 2 were controls. Sensitivity of these both

tests were 71.42%, specificity was 51.61%, predictive value for positive test was 10%, and predictive value for negative test was 96%. The percentage of false positive for both tests was 48.38% false negative was 28.57%. The two patients in control group with test positive results had no previous or present pregnancy fetal wastages. The 5 patients with recurrent pregnancy losses were positive for both the tests. Among the 5 cases, three had abortions, one had fetal death, and one delivered a live child during present pregnancy. The Incidence of lupus anticoagulant antibody in our study was 10%. The relative risk for pregnancy loss due to LAC was 1.4 [i.e., there was 1.4 fold increased risk for fetal loss with LAC].

All the patients (both cases and controls) had negative results for IgM assay for anticardiolipin antibody.

Table-7 shows that eight patients were positive for anticardiolipin test with IgG assay, of which 7 were study cases, 1 was in control group. Sensitivity of the test was 87.5%, specificity was 53.26%. Predictive value for positive test was 14% and predictive value for negative test was 98%. The percentage of false positive was 46.73% and false negative was 12.5%.

The patient in control group had no pregnancy wastage. Among the 7 cases, three had abortions, one had fetal death, and three delivered live children. The Incidence of anticardiolipin in our study was 14%. The relative

risk for pregnancy loss with ACL was 1.8 [i.e., there was 1.8fold increased risk of fetal loss with ACL].

Creagh et al (1991) studied 35 women with recurrent pregnancy loss and found 20%incidence for lupus anticoagulant and 17.1%incidence for anticardiolipin antibody.

MacLean et al (1994) studied 243 women and reported an incidence of 6.6%for lupus anticoagulant and 8.2%for anticardiolipin antibody.

Kumar et al (2002) studied 150 women and the incidence in the study was 10.28%for lupus anticoagulant and 40.24% for anticardiolipin antibody.

Velayuthaprabhu et al (2005) studied 155 women and the incidence was 14% for lupus anticoagulant and 40%for anticardiolipin antibody.

Mishra et al (2007) studied 120 women and reported an incidence of 15%for lupus anticoagulant and 28.3%for anticardiolipin antibody.

The incidence of lupus anticoagulant in our study was 10% and for anticardiolipin antibody, it was 14%.

Comparison with Previous studies

Studies	No. of patients	LAC %	ACL%
Creagh et al 1991	35	20	17.1
MacLean et al 1994	243	6.6	8.2
Kumar et al 2002	150	10.28	40.24
Velayuthaprabhu et al 2005	155	14	40
Mishra et al 2007	120	15	28.3
Present	50	10	14

Comparing the test for anticardiolipin using ELISA with test for lupus anticoagulant using coagulation tests, test for ACL had a sensitivity of 62.5%, specificity of 97.82%, and predictive value for positive test of 71.42%, predictive value for negative test of 96.77% (table-8). The predictive value of the test for anticardiolipin can be improved with combining both the tests.

Table-9 shows the pregnancy wastages in previous pregnancy among all cases. There were 74.49% of abortions and 21.47% of fetal deaths.

Table 10 shows the number of abortions in previous pregnancy of all cases. Ten percentages had one prior abortion, 54.55% had two prior abortions, 32.65% had three previous abortions and two percent had 4 abortions.

As shown in table-11, the present pregnancy outcome was compared between all cases and controls. Forty six patients in the study group gave birth to live children, four had fetal losses among them, and all 50 in control group gave birth to live children(p = 0.041).

The results showed the significant association between pregnancy outcome and presence of lupus anticoagulant and anticardiolipin antibodies.

Table-12&13:

In the present pregnancy, among LAC positive cases, 60% had abortions, 20% had live births, another 20% had fetal deaths (p = 0.08).Among ACL positive cases, 42.85% had abortions ,14.28% had fetal deaths and 42.85% delivered live children(p=0.154).

As shown in table -14, in the present study 42.8 % were second trimester losses and 14.2% of losses were in third trimester.

Chakrabarthi's study reported that 73.1% were first trimester losses, 26.9% were second trimester losses.

Thind et al reported 23% abortions, 57.3% intrauterine deaths.

Branch et al, reported 50-60% of losses in first trimester, 30% in second trimester, 10-28% in third trimester.

As shown in table-15, among the seven antiphospholipid antibody positive cases 72.72% were abortions, 22.72% were fetal deaths and 4.5% were live births during previous pregnancy. During present pregnancy, 42.85% were abortions, 14.28% were fetal deaths and 42.85% were live births.

Table-16&17 shows that out of 22 previous pregnancies, 11 had preeclampsia, ($p = 0.00$), 16 had abortions, ($p = 0.00$), 2 had intrauterine growth restriction ($p = 0.005$), 2 had abruption ($p = 0.005$), and 5 had intrauterine fetal deaths ($p = 0.00$). The incidence of these obstetric complications had a significant association with antibody positivity. The incidence of preterm labour was 1.34% in all cases, 4.54% in positive cases ($p=0.05$).

In present pregnancy, 3 cases had abortions, 2 had preeclampsia, and 1 had an intra uterine death of fetus, 1 had preterm labour and another one delivered a growth retarded baby. There were no incidences of abruption in the present pregnancy.

The present study shows that lupus anticoagulant and anticardiolipin antibodies are significantly associated with repeated pregnancy losses.

All the 7 cases with antiphospholipid antibodies, once they were detected to be antibodies positive, they were put on heparin and aspirin treatment. The dosage was Injection Heparin 5000units subcutaneously twice daily along with Tablet Aspirin 75mg once daily. The outcome was as follows: out of the 5 patients detected in second trimester, 3 had abortions, 2 had live births. Out of the 2 detected in third trimester, 1 had fetal death, 1 had live birth. Since our study was mainly a screening test, the details of treatment were not taken in to account for the evaluation of results and outcome. However counseling was given and patients were advised to report as soon as they miss the period in future pregnancy.

Women with lupus anticoagulant and anticardiolipin antibodies have a significant risk of reproductive failure, and adverse pregnancy outcome like recurrent abortions, intra uterine fetal death and intra uterine growth retardation. Women with history of thrombosis, or unexplained pregnancy loss should be screened for lupus anticoagulant and anticardiolipin antibodies along with maternal and fetal surveillance for a successful pregnancy outcome.

SUMMARY

SUMMARY

The present study was a prospective study aimed at screening for the presence of lupus anticoagulant and anticardiolipin antibodies in 50 patients with history of recurrent pregnancy losses and 50 controls matched for age, gestational age at study, of proven good reproductive history.

The distribution of study cases were 10% in first trimester, 76% in second trimester, and 14% in third trimester.

The tests done were Activated Partial Thromboplastin Time and diluted Russell Viper Venom Time for lupus anticoagulants and ELISA for anticardiolipin antibodies.

Five cases (10%) were positive for lupus anticoagulant, seven cases (14%) were positive for anticardiolipin antibodies. Hence the incidence of LAC and ACL in our study population was 10% and 14% respectively. The relative risk for pregnancy loss due to lupus anticoagulant was 1.4 and for anticardiolipin antibody, the relative risk was 1.8.

Both APTT and dRVVT tests were sensitive in detecting lupus anticoagulant antibodies, but test for anticardiolipin had more specificity in our study.

The outcome of LAC positive cases during previous pregnancy was as follows: 64.7% were abortions, 29.41% were fetal deaths, and 5.88% were live

births. During present pregnancy 60% were abortions, 20% were fetal deaths, 20% were live birth.

The outcome of ACL positive cases during previous pregnancy was as follows: 72.72% were abortions, 22.72% were fetal deaths and 4.5% were live birth. During present pregnancy 42.85% were abortions, 14.28% were fetal deaths, 42.85% were live births. Other pregnancy related complications noted were:

a) Preeclampsia; 50% of cases had preeclampsia in their previous pregnancy, 40% of cases had during present pregnancy.
b) IUFD: 22.72% of cases had intra uterine fetal death during previous pregnancy and 20% of cases had intrauterine fetal death during present pregnancy.
c) IUGR: 9% of cases had growth retarded babies in previous pregnancy and one had growth retarded baby during present pregnancy.
d) Preterm labour: 4.54% of cases had preterm labour during previous pregnancy and one had it during present pregnancy.
e) Abruptio placenta: 9% of cases had abruption during previous pregnancy and none had abruption during present pregnancy.

CONCLUSION

CONCLUSION

The presences of antiphospholipid antibodies [lupus anticoagulant and anticardiolipin] are associated with adverse pregnancy outcome. Patients with recurrent pregnancy loss need to be subjected to investigations for these antibodies.

Identification of those patients early, with early institution of therapy will help in minimizing the associated complications and pregnancy losses, thus aiding the anxious mother in delivering a live child.

The commonly used tests for detection of these antibodies are activated partial thromboplastin time and dilute Russell`s viper venom time for lupus anticoagulant and ELISA for anticardiolipin since performing more than one lupus anticoagulant screening test is important for optimal sensitivity. Moreover the predictive value of testing with anticardiolipin ELISA can be improved to an extent by concurrent lupus anticoagulant testing.

In combination, these tests are sensitive, specific which can be used in conjunction to screen the patients with recurrent pregnancy loss and are mandatory in the investigation protocol for these patients in order to prevent further pregnancy wastages.

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PROFORMA

PROFORMA

SCREENING FOR LUPUS ANTICOAGULANT ANTIBODY AND ANTICARDIOLIPIN ANTIBODY IN RECURRENT PREGNANCY LOSS.

Name :

Age :

IP No :

Unit :

Gravida :

Para :

Live :

Abortion :

Socioeconomic status :

Education :

L.M.P :

E.D.D :

Gestational age :

Booked/Immunised :

Menstrual history :

Age at menarche :

Periods :

Marital history :

M/S :

Consanguinity :

Obstetric history :

Past :

Present :

Risk factors :

H/O hypertension :

H/O drug intake :

H/O diabetes :

H/O Rh incompatibility :

H/O SLE :

H/O autoimmune

Disorders :

Past history :

Medical :

Surgical :

Personal history :

Family history :

General examination :

Built :

Nourishment :

Anaemia :

Pedal edema :

Jaundice :

Goiter :

Breast :

Spine :

Features of SLE/

Autoimmune

Disorders :

Height :

Weight :

BMI :

Vital signs :

Temp: PR:

RR : BP:

Systemic examination :

CVS :

RS :

CNS :

P/A :

P/V :

Investigations :

Urine albumin :

Sugar :

Deposits :

C&S :

Complete Haemogram :

Hb :

RBC :

PCV :

TC :

DC :

PLATELETS :

Bleeding time :

Clotting time :

Blood urea :

Sugar :

Serum creatinine :

Uric acid :

Proteins :

Plasma fibrinogen :

VDRL :

HIV :

Blood group&type :

USG abdomen :

Endocrinologist`s

Opinion :

Diabetologist `s opinion :

Geneticist`s opinion :

LAC result

APTT :

dRVVT :

ACL result

IgM :

IgG :

MASTER CHART

MASTER CHART FOR CASES

S.No	Name	Age	I.P.No:	Parity	G.A	LAC tests				aCL test		Pregnancy Outcome
						APTT		dRVVT		IgM	IgG	
						Study	Control	Study	Control			
1	Selvi	26	12326	G3P0L0A2	18	33	35	35	33	Absent	Absent	LB
2	Ayesha	31	13777	G4P1L0A2	18	34	37	35	32	Absent	Absent	LB
3	Nathiya	18	13806	G4P1L0A2	22	38	40	32	34	Absent	Absent	LB
4	Gousiabegum	22	13912	G5P1L1A3	11	34	32	34	36	Absent	Absent	LB
5	Maheswari	27	14111	G3P1L0A1	24	35	38	36	34	Absent	Absent	LB
6	Daisy Raju	34	14214	G4P2L0A1	24	33	31	34	31	Absent	Absent	LB
7	Aathilakshmi	24	12287	G5P2L0A2	14	54	35	52	36	Absent	Present	A
8	Senthamari	28	15101	G4P1L0A2	28	36	37	30	32	Absent	Absent	LB
9	Anitha	32	14011	G4P2L0A1	23	36	34	32	35	Absent	Absent	LB
10	Salima Banu	21	14927	G3P0L0A2	29	35	33	30	33	Absent	Absent	LB
11	Priya	21	14172	G3P0L0A2	25	32	35	34	36	Absent	Absent	LB
12	Jaya	25	13119	G4P1L0A2	28	34	38	34	32	Absent	Absent	LB
13	Gayathri	26	13911	G5P0L0A4	18	60	32	58	33	Absent	Present	A
14	Jothi	22	14221	G5P1L1A3	26	36	40	34	30	Absent	Absent	LB
15	Lakshmi	23	13992	G4P1L0A2	12	34	40	32	31	Absent	Absent	LB
16	Mumtaz	20	14217	G4P0L0A3	18	32	38	30	32	Absent	Absent	LB
17	Humera Begum	33	15105	G3P0L0A2	30	38	37	30	34	Absent	Absent	LB
18	Vembuli	29	13333	G3P2L0A0	28	52	32	55	36	Absent	Present	FD
19	Revathi	31	13897	G4P1L0A2	16	35	31	34	32	Absent	Absent	LB
20	Sasikala	27	14288	G4P0L0A3	19	30	32	35	33	Absent	Absent	LB
21	Glory	23	12927	G5P2L1A2	22	34	32	35	32	Absent	Absent	LB
22	Vimala	35	13827	G3P0L0A2	24	36	34	32	34	Absent	Absent	LB
23	Jaya Lakshmi	27	13914	G4P2L0A1	32	36	34	37	35	Absent	Absent	LB
24	Amutha	30	12906	G4P2L0A1	24	36	39	34	33	Absent	Absent	LB
25	Kamala	22	14111	G3P0L0A2	26	32	30	35	34	Absent	Absent	LB

26	Thirumala devi	21	12114	G4P0L0A3	16	34	32	35	31	Absent	Present	LB
27	Parveen	27	12346	G4P1L0A2	10	32	30	34	30	Absent	Absent	LB
28	Yasmin banu	22	13118	G5P1L0A3	18	36	31	35	36	Absent	Absent	LB
29	Karpagam	26	12916	G4P1L0A2	30	36	37	32	34	Absent	Absent	LB
30	Vimala	26	13416	G5P2L0A2	22	34	39	34	33	Absent	Absent	LB
31	Rani	29	12876	G4P0L0A3	24	38	40	35	32	Absent	Absent	LB
32	Rajeswari	24	14110	G5P1L1A3	32	34	32	36	35	Absent	Absent	LB
33	Rajmma	32	13191	G4P1L0A2	16	35	36	34	33	Absent	Absent	LB
34	Santhi	33	14625	G4P0L0A3	20	58	40	55	34	Absent	Present	A
35	Kavitha	23	14127	G4P0L0A3	26	36	38	32	36	Absent	Absent	LB
36	Kalyani	23	12356	G3P0L0A2	28	32	37	32	35	Absent	Absent	LB
37	Yogalakshmi	19	13671	G4P0L0A3	11	38	34	35	32	Absent	Absent	LB
38	Ammu	28	13238	G3P0L0A2	26	36	34	32	30	Absent	Present	LB
39	Anbarasi	21	12428	G3P0L0A2	33	32	33	34	31	Absent	Absent	LB
40	Alima	27	13761	G5P2L0A2	27	36	34	30	32	Absent	Absent	LB
41	RajaLakshmi	30	13573	G5P2L1A2	17	69	40	58	40	Absent	Present	LB
42	Saraswathi	22	14615	G5P2L0A2	18	36	32	32	34	Absent	Absent	LB
43	Santhimeena	26	14884	G4P0L0A3	16	35	32	34	32	Absent	Absent	LB
44	Pavithra	27	15111	G4P0L0A3	20	32	34	35	32	Absent	Absent	LB
45	Ponnamal	23	12500	G4P1L0A2	22	36	32	32	36	Absent	Absent	LB
46	Parimala	25	12715	G3P0L0A2	22	38	39	33	38	Absent	Absent	LB
47	Rooba	29	13428	G4P0L0A3	12	40	37	34	31	Absent	Absent	LB
48	Poovazhagi	24	12617	G5P1L1A3	20	36	32	32	34	Absent	Absent	LB
49	Ponni	28	12702	G3P0L0A2	26	34	32	30	33	Absent	Absent	LB
50	Prabha	24	13716	G4P0L0A3	30	38	40	35	34	Absent	Absent	LB

MASTER CHART FOR CONTROLS

S.No	Name	Age	I.P.No:	Parity	G.A	LAC tests				aCL test		Pregnancy Outcome
						APTT		dRVVT		IgM	IgG	
						Study	Control	Study	Control			
1	Ramya	21	12666	G2P1L1	20	34	32	35	34	Absent	Absent	LB
2	Kamala	29	12895	G2P1L1	24	31	35	32	32	Absent	Absent	LB
3	Yuvarani	24	12421	G2P1L1	24	35	37	32	36	Absent	Absent	LB
4	Ambika	30	12440	G3P2L2	28	56	36	52	37	Absent	Absent	LB
5	Aruna	17	12323	G2P1L1	18	34	37	32	38	Absent	Absent	LB
6	Devi	28	12682	G2P1L1	16	35	35	34	35	Absent	Absent	LB
7	Deepa	27	12445	G2P1L1	28	36	34	36	37	Absent	Absent	LB
8	Anjalai	26	12544	G2P1L1	14	32	35	38	36	Absent	Absent	LB
9	Bagya	31	12899	G2P1L1	22	34	32	36	38	Absent	Absent	LB
10	Chitra	23	12545	G2P1L1	28	35	34	32	35	Absent	Absent	LB
11	Shenbegum	34	12684	G3P2L2	11	34	31	34	37	Absent	Absent	LB
12	Seetha	24	12665	G2P1L1	26	35	32	36	38	Absent	Absent	LB
13	Radha	25	12768	G2P1L1	24	35	34	35	37	Absent	Absent	LB
14	Nirmala	32	12678	G2P1L1	29	34	35	35	34	Absent	Absent	LB
15	Amudha	22	13918	G2P1L1	18	34	37	35	33	Absent	Absent	LB
16	Barani	26	12889	G2P1L1	16	33	36	35	32	Absent	Absent	LB
17	Kalaiselvi	35	13137	G4P3L3	14	35	38	34	30	Absent	Absent	LB
18	Tamilselvi	19	13158	G2P1L1	18	35	32	38	40	Absent	Absent	LB
19	Sundari	28	12679	G2P1L1	32	54	34	52	38	Absent	Absent	LB
20	Thangamani	29	13188	G2P1L1	22	34	33	35	37	Absent	Absent	LB
21	Stella	21	12892	G2P1L1	20	34	31	34	36	Absent	Absent	LB
22	Bhavani	27	13199	G2P1L1	20	37	30	34	37	Absent	Absent	LB
23	Sivagami	26	12680	G2P1L1	24	38	40	32	35	Absent	Absent	LB
24	Sankari	23	13200	G2P1L1	32	38	38	35	34	Absent	Absent	LB
25	Amsa	31	12688	G2P1L1	24	35	37	34	32	Absent	Absent	LB

26	Geetha	22	12919	G2P1L1	10	35	32	35	33	Absent	Absent	LB
27	Prema	26	14811	G2P1L1	16	34	31	34	35	Absent	Absent	LB
28	Selvi	21	14872	G2P1L1	30	36	34	36	37	Absent	Absent	LB
29	Jayanthi	25	12877	G2P1L1	18	35	33	34	36	Absent	Absent	LB
30	Dhanalakshmi	26	15112	G2P1L1	18	35	34	35	37	Absent	Absent	LB
31	Sumathi	24	12927	G2P1L1	24	32	35	36	38	Absent	Absent	LB
32	Pushpa	23	14119	G2P1L1	28	34	36	35	33	Absent	Absent	LB
33	Alamelu	27	13976	G2P1L1	12	36	37	35	34	Absent	present	LB
34	Maha	27	12999	G2P1L1	26	37	38	34	35	Absent	Absent	LB
35	Sudha	22	12690	G2P1L1	26	35	34	34	32	Absent	Absent	LB
36	Megala	24	12222	G2P1L1	30	32	35	35	33	Absent	Absent	LB
37	lakshmi	29	13999	G3P2L2	28	35	33	35	34	Absent	Absent	LB
38	Kala	29	12976	G2P1L1	22	36	34	38	36	Absent	Absent	LB
39	Nirosha	27	12333	G2P1L1	24	34	32	36	38	Absent	Absent	LB
40	Raji	18	12787	G2P1L1	28	35	31	37	39	Absent	Absent	LB
41	Sundara	33	13912	G3P2L2	26	36	34	38	40	Absent	Absent	LB
42	Valli	23	13334	G2P1L1	12	35	32	35	33	Absent	Absent	LB
43	Dhiya	32	12114	G2P1L1	22	32	34	34	32	Absent	Absent	LB
44	Kavya	28	13378	G2P1L1	32	35	33	34	35	Absent	Absent	LB
45	Sumathi	31	12771	G3P2L2	20	34	32	36	37	Absent	Absent	LB
46	Ganga	30	13414	G2P1L1	18	30	34	34	36	Absent	Absent	LB
47	Vanitha	21	13578	G2P1L1	16	33	31	35	37	Absent	Absent	LB
48	Gowri	26	12116	G2P1L1	24	32	30	35	38	Absent	Absent	LB
49	lalitha	22	12888	G2P1L1	28	35	32	34	34	Absent	Absent	LB
50	sindhu	25	13771	G2P1L1	34	35	33	35	33	Absent	Absent	LB

ABBREVIATIONS

ACL- Anticardiolipin

ACOG- American College of Obstetricians and Gynecologists

APS- Antiphospholipid antibody syndrome

APTT- Activated partial thromboplastin time

dRVVT- dilute Russell`s viper venom test

EIA- Enzyme immuno assay

ELISA- Enzyme linked immuno sorbent assay

Ig- Immunoglobulin

IUFD- Intrauterine fetal death

IUGR- Intrauterine growth restriction

KCT- Kaolin clotting time

LAC- Lupus anticoagulant

RCOG- Royal College of Obstetricians and Gynaecologists

RPL- Recurrent pregnancy

ETHICAL COMMITTEE CERTIFICATE

I, **Dr.S.Maheswari** apply for the ethical committee certificate for the project

"**SCREENING FOR LUPUS ANTI COAGULANT ANTIBODY AND ANTICARDIOLIPIN ANTIBODY IN RECURRENT PREGNANCY LOSS**" under the guidance of **Prof Dr.Abraham Isacc**,M.D.,D.G.O , Institute of Social Obstetrics and Govt KGH Chennai.

I understand the implications of doing research with human subjects and will fully comply with the regulations and keep the dignity and protect the health of subjects at all costs.

S. Mahi

Signature of Postgraduate student

I have no objection to guide this postgraduate student in the project mentioned above. I shall supervise that all the human rights are protected and research is carried on with the utmost humanitarian principles.

Senior Civil Surgeon
Institute of Social Obstetrics and
Govt. Kasturba Gandhi Hospital for
Women & Children, Chepauk,
Triplicane, Chennai-600 005

Signature of the guide **Seal of guide**

I certify that this project has been presented in front of the Ethical Committee, duly formatted in this institution and that all the members of the Ethical Committee have given permission to conduct this research.

Chairman of Ethical Committee **Seal of Chairman**
Date:

CHAIRMAN
ETHICAL COMMITTEE
CHENNAI
Date:- 21.12.2005

PATIENT CONSENT FORM

STUDY TITLE: " SCREENING FOR LUPUS ANTI COAGULANT ANTIBODY AND ANTICARDIOLIPIN ANTIBODY IN RECURRENT PREGNANCY LOSS "STUDY CENTRE: Institute of Social Obstetrics& Govt.K.G.H. Chennai-5

PARTICIPANT NAME: AGE: SEX: I.D. NO.

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction.

I have been explained about the possible complications that may occur during the procedure. I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving any reason.

I understand that investigator, regulatory authorities and the ethics committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I understand that my identity will not be revealed in any information released to third parties of published, unless as required under the law. I agree not to restrict the use of any or results that arise from the study.

I hereby consent to participate in this study of " **SCREENING FOR LUPUS ANTI COAGULANT ANTIBODY AND ANTICARDIOLIPIN ANTIBODY IN RECURRENT PREGNANCY LOSS** "

Signature of Investigator Place:

Date:

Study Investigators Name Institution:

Signature / Thumb impression of patient

Thanking you,

Yours faithfully,

Postgraduate

Guide:

Prof Dr.Abraham Issac ,M.D.,D.G.O ,

Institute of social obstetrics&Govt.KGH,

Madras Medical College,

Triplicane, Chennai- 600 005

சுய ஒப்புதல் படிவம்
ஆய்வு செய்யப்படும் தலைப்பு

மீண்டும் மீண்டும் கருசிதைவு ஏற்படும் பெண்களில் ரத்தத்தில் லுப்பஸ் ஆண்ட்டி கொயக்லன்ட்

ஆய்வு செய்யப்படும் இடம் : சமூக மகப்பேறியில் நிலையம் மற்றும் அரசு கஸ்தூர்பா காந்தி மருத்துவமனை, சென்னை.

பங்கு பெறுபவரின் பெயர் :

பங்கு பெறுபவரின் எண் :

பங்கு பெறுபவர் இதனை (✓) குறிக்கவும்

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிகப் பட்டுள்ளது என்பதை அறிந்து கொண்டேன்.	
நான் இவ்வாய்வில் தன்னிச்சையாகத்தான் பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த சட்ட சிக்கலுக்கும் உட்படாமல் என்னை இவ்வாய்வில் இருந்து விலக்கி கொள்ளலாம் என்றும் அறிந்து கொண்டேன்.	
இந்த ஆய்வு சம்மந்தமாகவோ, இதை சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும்போது இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என் மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிகொண்டாலும் இது பொருந்தும் என அறிகிறேன்.	
இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ, முடிவையோ பயன்படுத்திக்கொள்ள மறுக்கமாட்டேன்.	
இந்த ஆய்வில் நான் பங்குகொள்ள ஒப்புக்கொள்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதியளிக்கிறேன்.	

பங்கேற்பவரின் கையொப்பம்.....

இடம்:.....தேதி.....கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்.....

ஆய்வாளரின் கையொப்பம் .....இடம் .....தேதி.....

ஆய்வாளரின் பெயர்.....